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The Grand Hotel, Brighton
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LABORATORY TECHNIQUES TO DETERMINE, FOR ADVISORY PURPOSES, THE SUSCEPTIBILITY OF THE FRUIT TREE RED SPIDER MITE TO ACARICIDES

by P. G. Clinch
Lenton Experimental Station

Summary

Reports of the failure of acaricides to control the Fruit Tree Red Spider mite are common but without definite evidence of resistance it is often difficult to know which material to advise growers to use. Laboratory techniques are described which enable the effectiveness of field concentrations of acaricides to be compared against any strain of Fruit Tree Red Spider mite. Examples of such tests are given, together with the results of recommendations following them.

Introduction

During the past four or five years many fruit growers in England have reported that certain acaricides, which up to that time had successfully controlled the Fruit Tree Red Spider mite, now failed to do so. In the early 1950's parathion was used to control the mite but because of this material's high mammalian toxicity growers quickly turned to chlorfenson and chlorbenside when these were introduced. In 1955 chlorfenson failed to give adequate control in some orchards in Essex and in the following year more growers used chlorbenside. In that year the majority of users were satisfied with the material although some lack of adequate control was reported, again from Essex. Since 1957 reports of failure with chlorbenside and other acaricides have occurred.

So far there have been no cases recorded of the failure of tetradifon in the United Kingdom. This may be due to the fact that this material is relatively new to this market and has not therefore been in general use long enough to select resistant strains. Resistance to this compound has, however, already been reported in the United States (Asquith, 1961).

Suggestions as to the cause of these failures have been put forward, and include, either individually or collectively, incomplete spray cover, incorrect timing, lack of predators, "hard leaf" and "the weather". Very little evidence has been produced to show that the build-up of resistant strains of the mite is the cause, although Collyer and Kirby (1959) did report one case in Essex.

Three courses are open to the advisory entomologist in cases where difficulty in controlling the mite has been experienced and resistance is suspected:- he can (1) recommend that the suspect material is re-applied and hence risk a wastage if the failure is confirmed; (2) advise the use of a new material and risk failure for the development of resistance to one material may also result in resistance to another; or (3) carry out tests with samples of the mite population in question and determine its suscept-

ibility to a range of acaricides so that one can be recommended without risk. It was with this latter course in mind that the work described below was undertaken.

This paper is not intended to be a survey of the resistance problem in the United Kingdom, the results quoted being from selected sites chosen to illustrate how the techniques described can be employed when cases of difficulty in red spider control are being investigated.

The Test Plant and its Preparation

Leaves for test can be detached from trees growing in the open but are available only in the summer months, though this period can be extended if apple seedlings are used. The technique adopted to obtain the seedlings was similar to that described by Massee (1947) but it was found, that the germination of the seed is improved if, after they have been sown in the boxes, they are subjected to a period of at least 40 days in a cold store at a temperature just above freezing point.

Several boxes of apple seeds are retained in the cold store, one being removed at intervals, the seeds allowed to germinate and grow to the 4 leaf stage undisturbed to develop satisfactory roots. The plants are then transplanted into small tubes containing nutrient solution and the leaves reduced to two in preparation for treatment. By this method a large number of leaves can be obtained quickly.

Rootstocks can also be used to provide leaves and the one tested (M. M. 106) is satisfactory except that it is very susceptible to Mildew.

Leaves can be used either detached from or attached to the plant. Detached leaves remain alive for at least a week provided they are removed with a piece of stem attached (a technique similar to the preparation of leaf cuttings) and the petioles are immersed in water. The choice of leaves is important as there is a tendency for those of large size or soft texture to wither. As an even cover of the chemical on the leaves is preferable, spraying to a stage before run-off is more satisfactory than dipping. Detached leaves can be sprayed in a Potter Tower (Potter 1952) or a similar apparatus, although to obtain an overall cover the upper and lower surfaces have to be treated separately.

Leaves can be treated attached to the plant if apple seedlings or stocks are used. If there are several leaves these are reduced to two on opposite sides of the stem to avoid masking. The plant is sprayed in a cabinet from two jets, similar to those designed by Howlett (1946), situated above and below the plant which is placed on a turntable and spray is applied as the plant rotates so that both the upper and lower surfaces of the leaves are treated simultaneously. By using a suitable support detached leaves can treated in a similar manner.

To determine accurately the L. D. 50 level of a compound, tests at several concentrations are required. For advisory work however, the tests must be as simple as possible and the compound must be sprayed at only one concentration, that recommended for high volume application

in the field which will usually give 100% mortality in the laboratory with susceptible strains. It can be assumed that if the kill obtained is much less than this in two consecutive tests, then the strain is resistant. As far as possible a strain of mites known to be susceptible to the materials under test is included for comparison. Not less than four replicate leaves are used for each compound.

Test Methods and Results

I. Using Adult Mites

If attached leaves are used, a petroleum jelly barrier may have to be employed to prevent the mites migrating down the stem.

a) To determine the susceptibility of eggs and larvae

Apple leaves, with the petioles in tubes of water, are infested by means of a fine brush with enough mites (10 - 20) to lay between 30 and 50 eggs in 24 hours, when subsequently stored in a constant temperature room at 24° C and 60% relative humidity. The mites are then removed with a fine glass tube attached to a vacuum source, leaving the eggs which are all of similar age. The leaves are next sprayed with the test chemical on both surfaces, replaced in the tubes and returned to the constant temperature room. Here they remained under daylight fluorescent lights with a 16 hour day for a further 8 days, by which time the majority will have hatched. The leaves are then examined under a binocular microscope, and all stages of the mite, both dead and alive, counted.

A typical set of results from such a test against the strain of mite thought to be resistant to chlorbenside is given in Table 1. Five replicate leaves were used for each material.

Table 1 The susceptibility of the summer eggs and larvae of a strain of Red Spider to acaricides

Treatment	Rate/100 gallons	No. of larvae alive	No. of eggs and larvae dead	Percentage mortality	Corrected mortality *
Phenkapton	16 fl.oz.	0	144	100	100
Tetradifon	25 fl.oz.	2	165	99	99
Chlorbenside	20 fl.oz.	75	103	58	54
Untreated	-	144	14	9	0

* Corrected for mortality in the untreated using Abbott's Method (1925)

The results of the test confirmed that the strain was resistant to chlorbenside. Phenkapton and tetradifon were included to determine whether they would be effective. The results confirmed that despite the resistance to chlorbenside, both phenkapton and tetradifon would be satisfactory.

b) To determine the susceptibility of adult mites

The technique is similar to that described above except that the mites are not removed at the end of the first 24 hours and assessment is carried out 48 hours after treatment.

The techniques described above are satisfactory when a large number of adult mites is available but when numbers are limited or the mites have to be transported long distances from the field to the laboratory, they are unsatisfactory. Because of this a method was developed to compare the susceptibility of larvae emerging from winter eggs. Although this method is suitable only for acaricides having persistant larvicidal action, it has the advantage that the susceptibility of the mites is known before any sprays are applied in the field.

II Method using Winter Eggs

Winter eggs can be collected in the autumn, winter and spring months, stored in polythene bags in a refrigerator or outside in the open (before the end of April) so that strains can be collected at different times and from different localities and tested together. Because of diapause the eggs cannot be tested before the end of March, (Lees 1953). After this time hatching is accelerated by placing the eggs in a constant temperature room at 24° C and 60% relative humidity, about 1 week before the test is due to commence. The time to hatch is dependent on a number of factors including the storage treatment received and may vary from one sample to another. When hatching has begun suitable apple leaves are sprayed with the test acaricide and placed in tubes of water. A piece of bark bearing approximately 200 unhatched eggs is then placed on each leaf so that the eggs are not in direct contact with it. The leaves are then placed in a constant temperature room where they remain under daylight fluorescent lights with a 16 hour day for 6 days. At the end of this time they are examined under a binocular microscope, the numbers of all dead stages and all alive stages except larvae being counted. Larvae still alive are ignored as they may have emerged too recently to be affected by the chemical.

Results

a) 1960

This method was developed early in 1959 and in the autumn and winter of that year wood bearing winter eggs was collected from five specially selected orchards where the growers had used chlorbenside or demeton-methyl for several years. In three of these, resistance to chlorbenside was suspected, in another resistance to both chlorbenside and demeton-methyl, whilst in the remaining orchard the mites were presumed susceptible to all materials, chlorbenside having been used for several years and given satisfactory control. In April 1960 the eggs from the five sites were tested as described using demeton-methyl, phenkapton, chlorbenside and tetradifon. The results given in Table 2 are the mean of two tests using four replicates per treatment in each test.

Table 2 Susceptibility of larvae hatching from winter eggs

Treatment	Rate/100 gallons	Percentage Mortality				
		Orchard I	Orchard II	Orchard III	Orchard IV	Orchard V
Demeton-methyl	12 fl.oz.	6	81	100	99	100
Phenkapton	16 fl.oz.	98	100	100	100	100
Chlorbenside	20 fl.oz.	42	46	70	66	93
Tetradifon	25 fl.oz.	100	99	100	100	97

The programmes adopted in the field and the results obtained are given below.

Orchard I (Essex) The grower failed to obtain satisfactory control of the mite with chlorbenside in 1958 and demeton-methyl in 1959. In 1960, after studying the laboratory test results, tetradifon was recommended and one application gave good control for the whole season.

Orchard II (Essex) Adequate control of the mite could not be achieved with chlorbenside in 1958 although demeton-methyl and malathion gave control in 1959. In 1960 the grower decided to spray two adjacent areas, one with chlorbenside at Pink Bud and the other with tetradifon at Petal Fall. The chlorbenside application was repeated again at the end of May. Counts taken on the 14th June 1960 are given in Table 3.

Table 3 Red Spider infestation at Orchard II

Treatment	Mites per 100 leaves		
	Adults	Nymphs & larvae	Eggs
Tetradifon	0	13	102
Chlorbenside	384	560	6704

Azinphos-methyl was applied to the chlorbenside plot after this count had been taken and gave satisfactory results.

Orchard III (Nottinghamshire) Chlorbenside did not give satisfactory control in 1959. In 1960, phenkapton was recommended and the trees receiving this material remained clean for the whole season. Trees sprayed with chlorbenside at Pink Bud had heavy infestations and by mid-June the number of mites present on these trees did not differ significantly from the untreated. Both the untreated and the chlorbenside treatment showed bronzing of the leaves early in the summer.

Orchard IV (Nottinghamshire) The control given by chlorbenside in 1959 was unsatisfactory and in 1960 phenkapton was recommended. This gave excellent control whilst trees sprayed with chlorbenside were bronzed by the end of June.

Orchard V (Worcestershire) No resistance problem existed in this orchard and any of the materials tested would have given good control. Phenkapton was in fact used and proved satisfactory.

b) 1961

A series of laboratory tests similar to those of 1960 using the same compounds and concentrations was made in 1961. The winter eggs were taken from five selected orchards, two of which were not used in the 1960 tests. The results given in Table 4 are the mean of two tests using four replicates per treatment in each test.

Table 4 Susceptibility of larvae hatching from winter eggs

Treatment	Rate/100 gallons	Percentage Mortality				
		Orchard I	Orchard II	Orchard III	Orchard IV	Orchard V
Demeton-methyl	12 fl.oz.	33	39	100	100	100
Phenkapton	16 fl.oz.	95	97	100	100	100
Chlorbenside	20 fl.oz.	21	22	20	12	32
Tetradifon	25 fl.oz.	100	100	100	100	98

The programmes adopted in the field and the results obtained are given below.

Orchard I (Essex) (Orchard I in the 1960 test) The results of the laboratory tests were similar in the two years. Despite the fact that neither chlorbenside nor demeton-methyl was applied the mites remained resistant to these materials. Tetradifon was used again in 1961 and as in 1960 gave good control.

Orchard II (Essex) (Orchard II in the 1960 test). The laboratory results for the two years are different, the mites having become less susceptible to demeton-methyl despite tha fact that this material was not used.

This may be due to the azinphos-methyl spray applied in 1960 for both are organo-phosphorus compounds and there is evidence that resistance developed to one such compound confers some degree of resistance to another. In 1961 tetradifon was applied and gave good control of the mite.

Orchard III (Essex) Chlorbenside was used in 1958, demeton-methyl in 1959 and tetradifon in 1960. Tetradifon was again used in 1961 and gave satisfactory control.

Orchard IV (Nottinghamshire) (Orchard IV in the 1960 test). The laboratory results for the 2 years are similar. Phenkapton again gave excellent control and chlorbenside, where applied, was unsatisfactory.

Orchard V (Worcestershire) Chlorbenside was used in 1960 followed by azinphos-methyl and the control obtained was not adequate. After carrying out the laboratory tests phenkapton was recommended in 1961. However, the grower again applied chlorbenside which failed to give control and by the end of June the foliage was bronzed.

Discussion

The results indicate that the laboratory tests described can give a useful indication as to whether or not an acaricide is likely to be satisfactory in the field. The orchards chosen for the tests were those where difficulty had been experienced in controlling the mite, and the results given are not intended to give a representative picture of the susceptibility of mites throughout the country to the acaricides mentioned.

At the moment most growers are obtaining satisfactory control of the mite with the materials available. However, in some areas a resistant strain may build up very quickly even after using an acaricide for only two consecutive seasons. A rotation of acaricides is therefore often recommended, and the more materials in the rotation the less likely is the selection of resistant strains.

It has already been reported that resistance developed to one organophosphorus may also confer some degree of resistance to another. The same suggestion has been made of chlorinated hydrocarbon acaricides having similar chemical structures and comparable types of activity, e.g. summer ovicides. It is interesting to note therefore that where resistance has developed to one organo-phosphorus material, demeton-methyl, another, phenkapton, could have been used satisfactorily. Only by testing each strain individually can a satisfactory rotation be devised.

Acknowledgements

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RESISTANCE OF THE FRUIT TREE RED SPIDER MITE (METATETRANYCHUS ULMI) TO ACARICIDES AND THE CONTROL OF RESISTANT STRAINS IN THE NETHERLANDS

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Introduction

The intensive applications of acaricides in Dutch apple orchards against the Fruit Tree Red Spider Mite (Metatetranychus ulmi) has led to resistance of many populations to various acaricides. In the south-western part of the Netherlands resistance to organo-phosphates is most common, while these compounds also have been very intensively used against other pests than the Fruit Tree Red Spider Mite. In the central part of the Netherlands resistance to ovo-larvicides is more common as these materials have been used there frequently, for the grass beneath the trees is often used for grazing and hay.

Resistance against organophosphates was reported in Zeeland in 1953 (van de Vrie, 1954) and 1958 (van de Vrie, 1959); resistance against ovo-larvicides was reported in 1958 (van de Vrie, 1959). From field observations it was learned that after a period of about 5 years (i.e. 25-30 generations) intensive application of one type of an acaricide a certain degree of resistance can be expected.

Resistance against organophosphates

Field- and laboratory trials indicate that resistance to one of organophosphates is coupled with less susceptibility to all other acaricides of this group. There are however differences in the degree of control by these acaricides; some still provide a certain degree of control, while others do not. Comparison of the effectiveness at lower concentrations on both susceptible and resistant strains showed that there is already a certain degree of resistance even to the effective acaricides.

The strains resistant to organophosphates have the normal susceptibility to the ovo-larvicides and to Kelthane; in both field- and laboratory trials good results could be obtained at the normal concentrations. These observations lead to the conclusion that a so-called "group-resistance" is present in these strains.

Resistance against ovo-larvicides

A group-resistance to the ovo-larvicides seems to occur; in cases where chlorbenside failed to give satisfactory control, neither fenson nor chlorfenson give good results, tetradifon seems to have a somewhat different position, as in some cases a good result could be achieved where the other ovo-larvicides failed. However the position of tetradifon is not yet clear as there are also failures.

The strains resistant to ovo-larvicides still have the normal susceptibility to organophosphates and to Kelthane; so it seems that also in this case a "group-resistance" is present.

Effect of some fungicides on the mite population

Most fungicides which are used in the control of apple mildew (Podosphaera leucotricha) possess acaricidal properties. As these materials have to be applied during the growing season at frequent intervals, the acaricidal effect is very valuable in the control of resistant strains of mites. The materials used under Dutch conditions are dinocap and dinitro-sec.-butylphenyldimethyl acrylate (= "Acricid"). These materials can keep the mite population on a rather low level, provided they are applied at frequent intervals and a good control of the fruit mite generation is carried out. As soon as the applications are stopped the remaining mites often build up a high population-density in a short time.

Advised spray programs

In case of organophosphate resistance, the following spray program is advised. The first generation of the Fruit Tree Red Spider Mite should be controlled by the application of an ovo-larvicide either at full strength at the beginning of the flowering period or at half strength just before blossoming and at half strength immediately after petal fall. The method chosen depends on the number of winter-eggs present; if large, the danger of damage during the blossoming period is great and an application of an ovo-larvicide prior to blossoming is advisable.

During the summer months the frequent applications of the mildew-fungicides can keep the mite population under control, but as soon as these applications are ceased, an application of Kelthane is advised. This application will prevent the build-up on a new population and also the production of winter-eggs.

In cases of ovo-larvicide resistance, the first generation can be controlled by using organophosphates; the systemic acaricides will do extremely well just after petal fall. If many winter-eggs are present and damage during the flowering period is expected, an application of phenkapton will be valuable. During the summer months the mildew-fungicides will keep the mite population under control and as soon as the mildew-control is finished, an application of Kelthane is advised.

These spray programs involve three different types of spray chemicals and each chemical is used in its most suitable period. Frequent change during one season seems to be the most efficient way of preventing the development of resistant strains of the Fruit Tree Red Spider Mite.

Under Dutch conditions these spray schedules have been followed now for four successive years without any indication of resistance to the materials used.

Summary

In apple orchards in the Netherlands many mite populations have become resistant against acaricides. As the resistance seems to be a "group-resistance" frequent changes in the acaricides used seem to prevent the development of resistance. The inclusion of fungicides with acaricidal properties in the spray program enables the use of three different types of acaricides during one season.

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ASPECTS OF BIOLOGY OF IMPORTANCE IN TIMING SPRAYS AGAINST CODLING MOTH, FRUIT TREE TORTRICID MOTH AND FRUIT TREE RED SPIDER MITE

Dr. F. R. Tubbs, Chairman, stated:

First I would like to thank those who have delivered the papers and also any co-authors.

I think it would be a good idea if we first discussed apple aphis, tortrix and pear sucker and left a longer time at the end, say 15 or 20 minutes, for our discussion on codling moth and red spider.

Q. Mr. P. D. Scott

Has Mr. Webb actual experimental evidence that a pear sucker attack in the autumn of one year reduces the crop in the following year.

A. Mr. Webb

We have seen a number of cases where following upon a heavy infestation of pear sucker from a third generation which has built up at the end of the season with plenty of nymphs around the fruit bud in these orchards, there has been a considerable amount of flower buds which did not open or perhaps partly opened, causing reduction of blossoms in the orchard. We have not, as far as we know, seen this in an actual trial but a number of cases have been reported to us. Visible also, on the spurs, there is a lot of black honey-dew fungus indicating the amount of pear sucker infestation.

Q. P. de Pietri-Tonelli

Do the Carpocapsa larvae in your orchards enter the fruits immediately after hatching or do they move around for quite a long time before entering the fruit? This external, and possibly ectophagous, period of variable length might be important for the choice of the insecticide to be used, having the right mode of action.

A. Miss Gratwick

Under laboratory conditions the time taken for larvae to select the point of entry has varied from a few minutes to hours; it is usually less then one hour. During this period they wander over the surface of the fruit.

Mr. Carpenter commented:

We have also encountered this in the field. Very few of the eggs which we marked for our trial entered the apples on which they were laid. However, there have been cases where entry has been directly beneath the egg.

Q. Dr. F. R. Tubbs, Chairman.

I would like to ask Dr. Dicker whether he has found many orchards where the hatching period of fruit tree red spider mite was late, and whether poor control due to resultant bad timing could have led to the belief that resistant strains existed in such orchards?

A. Dr. Dicker

We have little information as most of our work is done at East Malling. A few cases have been reported by members of commercial firms, but the proportion of orchards in which late hatching occurs is probably very small indeed. We have put forward the suggestion that normal populations consist of various components which hatch at different times and, due to the timing of sprays, the early hatching components have been eliminated from occasional orchards.

There is experimental evidence that where chemicals have failed this is due to real resistance to the chemical rather than due to time of hatching.

Q. P. de Pietri-Tonelli

What do you think about starting to spray against the first generation of codling moth when you just see the first damaged fruits instead of looking at the adults or eggs?

As you know, this method has been developed on the Continent, in Switzerland (Baggiolini and Savary) in Germany (Kotte) and in Italy (Antongiovanni).

A. Dr. Dicker

In England control measures are directed only against the first generation. It is therefore important that the first spray should be applied by the time the earliest larvae are hatching; otherwise some may survive and give rise to a partial second generation. Dr. Chiswell finds that at least 50% of the early larvae enter at the calyx and no external symptoms are visible for at least a week after entry. In addition, levels of infestation are low at this period and growers could not be expected to find the very early entries. It is for these reasons that we use adult activity as a guide to timing of sprays.

With the exception of lead arsenate, all the chemicals in current use are toxic to the adult moth, and the carbamates and organo-phosphorus compounds are also ovicidal. Control will therefore be obtained against eggs and adults even if the spray is applied before any larvae have hatched.

Mr. Carpenter commented:

The early stages of light infestation can only be detected with difficulty even by systematic searches with trained observers. Even on sites where the subsequent infestation has been 40% there was only 1% occurring at the last week in June. (That is as measured by a check entry.) To expect the grower to find a 1% entry by non-systematic examination is unrealistic at this level, providing that the proportion of check and eye entry is roughly equal the difficulty of identifying eye entry does not add to the problem of early detection.

P. de Pietri-Tonelli conceded:

I agree. There could also be a big difference between one and another variety. It is different in this country.

Dr. J. T. Martin commented:

Efficient coverage is difficult to achieve even by careful large volume spraying and the nature of the formulation used plays a large part in determining the level and distribution of the deposit obtained. Adequate wetting is important in the bud-burst applications. With regard to acquired resistance of insects, this does not seem so important at present here as in some other countries. We should, however, heed all warnings and take steps in good time, by modifying spraying practices, to keep the problem in check.

Q. Mr. J. W. Bryden

Regarding the question raised by Dr. P. de Pietri-Tonelli, I have worked on codling moth for two years in the Mediterranean area. I can support his view that it is practical for growers to estimate spray timing by observing the first signs of larval entry. I also agree with the U. K. speakers that this would be impractical in this country. Mediterranean infestations are much higher than those in the U. K. Egg hatching in the U. K. rises more slowly to its peak than under Mediterranean conditions where a period of a week or so of slight hatching is followed by a sudden, very high, peak. Sprays applied within a week of observing first larval entries were found to achieve good results.

Observation from Dr. Serralach

An extract from the following publication of Esso Standard, of France: "Les cahiers du Departement Agricole d'Esso Standard" No. 4 - Juin 1957. "Les vers de la grappe. Nouvelle donnees biologiques. Methode rationnelle de lutte". was given at the IV International Congress of Crop Protection under the title "The use of the damage curve of a pest to assess the worth of a preventive-curative treatment".

This technique, developed for Cochylis and Eudemis may be applicable to codling moth.

Q. Mr. Ebert

Can you tell me the variety of apple from which your codling moth results were obtained?

A. Miss Powell

Mainly Cox but Worcester, Bramley and Laxton as well and some James Grieve.

Q. Mr. Van de Vrie

Under Dutch conditions the application of ovo-larvicides against the first generation of Metatetranychus ulmi is carried out either at full strength at the beginning of the blossom period, or at half strength before the blossom and at half strength at petal fall, to prevent damage by the mites during the blossom period. The method chosen depends on the number of winter-eggs present on the branches.

The frequent application of fungicides against apple mildew can keep

the mite population under control, provided the first generation is very well controlled by acaricides. The application of fungicides with acaricidal properties gives the possibility of changing to other materials so making the risk of resistance-development smaller.

A. Dr. Dicker

I agree with Mr. Van de Vrie, regarding his remarks about dinocap and red spider control. It is however, a fairly common experience in England to find that the level of red spider is kept low only during the period that sprays are applied and populations increase to injurious levels later in the summer.

Q. Dr. Tubbs, Chairman.

Is there any information on the toxicity of azinphos-methyl to predators?

A. Miss Powell

We have not very much evidence on this subject but there is less damage than with D.D.T.

CONTROL OF BOTRYTIS DISEASES

Chairman: T. Swarbrick
Scottish Horticultural Research Institute

Introduction

This session is to be devoted to a particular group of fungi, Botrytis. This makes it of particular interest to those of us from Scotland where we have something like 7,000 acres of raspberries concentrated in the Dundee, Perth Blairgowrie, Arbroath area. In addition we have a substantial strawberry growing industry. As you know, we have already made substantial progress in our work on the Virus diseases of these two crops, but practically no progress at all has been made towards reducing the very serious losses that can occur from Botrytis in epidemic years. There is always some loss of crop due to Botrytis. It may be 5 - 6 cwt of strawberries per acre and 6 - 7 cwt of raspberries but in epidemic years, which occur at fairly short intervals, the loss is one to one and a half tons per acre. The potential market for a suitable fungicide should be seen against the background of yields and values. An average crop of raspberries produced by a good grower is from $2\frac{1}{2}$ to 3 tons per acre and with raspberries £95-100 per ton this gives gross income of £300 per acre and a loss of some income in epidemic years of £100 per acre. While it is true that some of the standard fungicides will give some control they cannot be used because they make the fruit unacceptable to the public.

It is against this background that we wish you to examine the problem of Botrytis, the diseases it causes and the present state of means for its control under field conditions.

Discussion

Q. Mr. R. W. Marsh

How does the development of resistance to the dithiocarbamates differ from that to the chlorinated nitrobenzenes?

A. Dr. R. K. S. Wood

There are a number of important differences. In the first place, a large proportion of Botrytis cultures exposed to the vapours of the chlorinated nitrobenzenes or to dicloran develop resistance relatively quickly, within 10-14 days. Secondly, the resistance which develops is, in some respects, almost complete because resistant strains grow almost as quickly in the presence as in the absence of the fungicides. However, most resistant strains do not sporulate in the presence of the fungicides although they do so freely once the fungicide has been removed. But we have obtained some resistant strains which will sporulate in the presence of the fungicides.

Another difference is in the way in which the resistant strains are produced. Those resistant to dithiocarbamates were produced only after a relatively long period of training in which the fungi were exposed to increasing, but sub-lethal doses of the fungicides, whereas the other strains appeared "spontaneously" after growing for some time in the vapour of the fungicides.

Undoubtedly, however, the most striking difference is in the order of resistance to the fungicides. In fact, I know of no other group of fungicides used on plants where resistant strains are so little affected by the fungicides.

Q. Dr. J. T. Martin

Recently reports have been made of the production in plants of antifungal agents in response to infection. Does Dr. Wood think that this occurs following Botrytis attack?

A. Dr. R. K. S. Wood

I think there is some evidence that this happens. We have done some work in this connection with the disease of broad beans caused by Botrytis fabae, and obtained some evidence for the development in bean tissues of substances formed following the oxidation of phenols which reduce the activity of the pectic enzymes secreted by B. fabae. And, of course, there is the evidence from similar work which has been done at Long Ashton. Such substances are really formed as a response to infection; I believe that it is fair to say this because the fungus kills cells of the host thus permitting phenols in the cells to be acted upon by the polyphenolases of the host or the fungus - the substances then formed inactivate the pectic enzymes of the fungus.

Q. Dr. E. Evans

Could you please comment on what you consider to be the main factors which determine whether a given infection by Botrutis becomes aggressive? Is inoculum potential one of these factors?

A. Dr. R. K S. Wood

I believe it is important. For example if you take healthy lettuce leaves and put drops of water containing spores of B. cinerea on the surface there is a fair chance that only a limited, leaf-spot type of lesion will be produced. But if the spores are suspended in a dilute solution of a readily available carbohydrate such as glucose, a rapidly spreading, aggressive type of lesion will be caused. You can also increase the chances of getting an aggressive type of lesion by increasing the number of spores in the drop, and by increasing the water content of the leaves. But certainly the most striking effects are produced by readily available carbohydrates. Incidentally, even with the same types of treatment one can get aggressive and non-aggressive lesions on the same leaf.

Q. Dr. J. B. Loughnane

Can Dr. Wood say if the species of Botrytis which attacks onion foliage is the same as that which causes rot of the bulbs? In my experience onion foliage is frequently attacked by Botrytis especially when the crop is grown in soils deficient in potash.

A. Dr. R. K. S. Wood

My statement that B. allii does not grow profusely on plants in the field was based on remarks to this effect in the literature, and on my own observations on small plots at our field station. But I am entirely open to correction on this point. However, even if B. allii is common on growing plants, it still puzzles me where the fungus comes from in the first place, particularly when the crop is grown on land which has not carried onion crops for some time.

Q. Mr. D. Rudd-Jones

Dr. Jarvis has suggested that a systemic fungicide might be expected to provide the most effective control of Botrytis on strawberries and raspberries. Surely, with a disease that kills the host tissue quite rapidly, it is unlikely that a systemic compound will control the disease unless it is applied and has become systemically distributed in sufficiently high concentrations to provide control before infection occurs?

A. Dr. W. R. Jarvis

Yes, I think a systemic fungicide would only be of value if it is applied before infection and in this case that would mean applying it at the pre-blossom, closed bud stage. We have in fact tried one systemic fungicide which has been shown to be reasonably good in glasshouse crops but we have had very patchy success in field soft fruit crops. It was first applied at the time when the fungicides are normally applied i.e. at the petal-fall stage, and it would appear that this was too late to be effective, even assuming that a sufficient concentration was present.

Q.

Recent work in Oregon has shown that infection of strawberry first occurs at a very early stage in its development and that it then remains symptomless, often until it is ripe, the fungus mycelium remaining in a quiescent stage. Has Dr. Jarvis confirmed this, and is there a similar behaviour in raspberries?

A. Dr. W R. Jarvis

Yes, I have confirmed Powelson's observations in Scotland and shown it to be true to a much greater extent in raspberries. We are still working on the mechanism which holds the fungus in check. It does not appear to be the same that Dr. Wood has recently described in bean leaves, but is possibly bound up with some substance which is present in green fruit but not present in the ripe fruit. It operates in both strawberries and raspberries.

Q.

Could Dr. Jarvis say whether green fruit is always completely symptomless, and if so, is the tannin in green fruit acting as a fungistatic agent?

A. Dr. W.R.Jarvis

It is not quite true that green fruit is always symptomless; one can in fact rot green strawberries, but it is not easy. The rot occurs occasionally under field conditions but usually escapes notice because it is generally concealed beneath the calyx. It appears only under warm conditions with temperatures well over 20° C. Regarding the tannin content of the green fruit, it is quite true that strawberries do contain an appreciable amount of tannin but so far as I know there is as much tannin in ripe fruit as in green fruit. We may have a case such as Dr. Wood describes for potatoes. If you kill the tissue, the fungus will grow on this and on an extract made from green fruit quite readily. It appears possible that some physical mechanism operates in the fruit, or a chemical mechanism, or possibly a combination of both.

Q. Mr. R.G.Pawsey

Botrytis damage is sometimes important in forest nurseries, particularly on spruce. Almost invariably the damage by the fungus follows physical damage, usually by frost. The frequency of frost in forest nurseries makes it extremely difficult to control Botrytis damage by spraying with contact fungicides and we are very interested in the development of suitable systemic materials. Could Dr. Jarvis give more information on the systemic fungicide he mentioned?

A. Dr. W.R.Jarvis

The material is the antibiotic, griseofulvin, and this is the only one of its type used. The results were variable possibly because the time of application was wrong; the infection may have occurred before the fungicide was applied. This work was done before we really appreciated that this very early infection occurred almost as soon as the buds were open.

Q. Mr. R.G.Pawsey

Griseofulvin has been used in forest nursery experiments with poor results. Can anybody give information on the use of cycloheximide against Botrytis infection?

A. Dr. W.R.Jarvis

I have no personal experience of the material. It has been used in the States but only as a post-harvest treatment in order to preserve soft fruit in storage and transit. I do not know of any work in which it has been used in the field.

THE BIOLOGY AND CONTROL OF DISEASES CAUSED BY BOTRYTIS SPP.

by R. K. S. Wood
Imperial College of Science and Technology

It is difficult to assess the losses caused by Botrytis spp. because of the variety of the crops which are attacked, the nature of the diseases which are caused, and because the incidence and severity of these diseases depend so much upon the weather and the way in which the crops are grown. There can, however, be little doubt that in many years, Botrytis cinerea, or one of the allied species, is the cause of major loss of lettuce, broad and field beans, onions, raspberries, strawberries, and various bulbs and corms. It can also be very troublesome on many other crops, particularly glass-house ornamentals, and although few systematic studies have been made, B. cinerea is probably one of the main reasons why in many seasons we find it so difficult to harvest good quality seed in this country. The total losses in the United Kingdom each year must still amount to many hundreds of thousands of pounds in spite of the attempts which are made to reduce them by the use of fungicides.

The diseases may be conveniently divided into two groups. In the first are those in which the fungi responsible are relatively specialised in their parasitism and are sufficiently distinctive, particularly in their conidiophores and conidia, to be recognised as species distinct from B. cinerea. Thus we have B. allii and B. byssoidea mainly parasitic on Allium spp., B. fabae mainly on Vicia faba but also on other legumes, and B. polyblastis largely confined to Narcissus. A number of these species have a perfect, apothecial stage which is not uncommon under natural conditions. In the second group are the many diseases caused by the aggregate species B. cinerea which probably has a host range as wide as any other fungus we have in this country. The species has a large number of forms which differ considerably in a variety of ways. Further study of some of the more distinct of these may justify their elevation to specific rank. Forms recognised as B. cinerea have been shown to have Sclerotinia fuckeliana as the perfect stage but apothecia seem to be rare in the field. Most forms and species produce conidia abundantly on their natural substrates, though they often do so far less readily in culture. Sclerotia are common in the field and are almost always produced abundantly in culture. A proportion of the conidia remain viable under ordinary laboratory conditions for many months, and the sclerotia are also long lived. Microconidia, much smaller than the conidia, may also be produced freely and function sexually before apothecia are formed.

The various diseases have a number of features in common. Although most species can probably invade and become established in healthy parts of plants, they do so more readily when the tissue being invaded is senescent, moribund or dead. Most of the primary infections during the growing season are made by the germ-tubes of conidia. The progress of the parasite after invasion depends on the type of tissue, its condition, and the surrounding temperature and humidity. In massive invasions of

relatively large bulks of storage tissue the rate of advance of the fungus is largely determined by temperature and by the nature of the tissue. At suitable temperatures, some tissues, e.g. those of apples, are rotted quite quickly. In others the spread of the fungus is relatively slow and not nearly so rapid as might be expected from the growth rate of the fungus on killed tissue in vitro. Thus, although B.allii moves continuously through onions so that ultimately the bulb is destroyed, it nevertheless moves slowly and cannot be regarded as an aggressive parasite under normal conditions of storage. The rot in onions, as in other tissues in which the fungus grows relatively slowly, has not the typical characters of a soft rot. We are at present studying this as well as other aspects of neck rot of onions.

When Botrytis spp. attack leaves their progress again depends on the nature of the tissue and on temperature, but now it is often much influenced by the water content of the tissues and the relative humidity of the air around the plant. A high relative humidity and, probably, free water is necessary for invasion and establishment and unless humid conditions persist the lesion very often fails to develop and the fungus does not sporulate. This is well seen in the typical spot lesions found on broad bean leaves infected by B.fabae, and on tulip leaves infected with B.tulipae. Both resemble those caused by more typical leaf spot pathogens such as Ascochyta spp. and Septoria spp. The spot character of the lesions is lost under certain conditions, particularly when the leaves are kept in a very high relative humidity so that their moisture content is probably higher than usual. The pathogen then spreads rapidly to cause a soft rot upon which it sporulates freely at a later stage. At present we know very little about the mechanisms that regulate the two types of lesions but the problem is of considerable interest both academically and practically because generally it is the spreading, aggressive type which is the more damaging in the field.

Botrytis spp. often attack stems. When they are soft and succulent, as in broad bean, the course of infection resembles that in leaves, but in more woody stems the attack generally takes the form of a die-back from the top of the shoot or occurs as a lesion lower down. The fungus moves relatively slowly through the tissues, although it generally sporulates very freely on the parts that have been killed. The slow movement through the woody parts of the stems is readily understood because of the nature of the substrate which has to be destroyed, but it is rather puzzling why the fungus does not move more readily through the softer tissues outside the cambium.

Now that I have given a brief outline of the ways in which Botrytis spp. attack higher plants, I should like to say a few words about resistance to infection and colonization by these fungi. Although conditions can be found in which almost every type of plant will succumb to attack by one species of Botrytis or another, it is often surprisingly difficult to get aggressive lesions established on well grown plants in "normal" conditions of humidity and temperature. Although the fungus may become established in such

plants, the lesions remain very small. Very little indeed is known about this very common and important type of resistance which is readily broken by comparatively minor changes in the external environment of the host. It is unlikely to be structural in character, or to depend upon substances already present in the tissues and which directly retard the growth of the parasite in vivo. I think it more likely that it depends on substances which are formed in the plant as a result of infection, and which interfere not so much with the internal metabolism of the pathogen as with the various toxins and enzymes by which the pathogen damages and kills the host tissue. The progress of the lesion will be determined by the rate at which such substances are produced and how long they retain their activity. Resistance will depend upon how quickly inhibitors of these substances are formed in the diseased tissue. In a balanced system of this sort, it is easy to see that slight alterations in the condition of the host might have cumulative effects which would profoundly alter the progress of the lesion.

Coming now to disease control, somewhat different problems are presented by the diseases caused by the more specialised species and by B. cinerea, although the differences are of degree rather than kind. When the parasite has a restricted host range it is at least feasible that the disease in a particular area could be greatly reduced in severity or eliminated by growing crops in clean soil and by planting only healthy material, or material which has been treated by heat or with fungicides so as to make the parasites innocuous. On a small scale disinfection of contaminated soil with fungicides might be practical. With these diseases there is the advantage that at the beginning of the season the sources of the inocula can be known with some certainty and are not likely to be numerous provided reasonable precautions are taken. "Fire" of tulips, caused by Botrytis tulipae may be placed in this group because for all practical purposes it is confined to the genus Tulipa.

At the other end of the scale there are the diseases caused by B. cinerea. Many forms of this species which are important pathogens will have a wide host range among cultivated and wild species present in areas where crop plants are likely to be grown. This make the problem of control much more difficult because no matter how much care is taken to reduce the amount of fungus associated with the crop plant, there will always remain a reserve of inocula in the environment around the crop which might attack the crop when conditions become suitable. And, of course, not much that is practical can be done to reduce their number. So even if the crop is planted on clean soil and the planting material is free from the fungus, one must assume that the crop will be attacked when temperature and humidity are suitable.

While it is easy to see why B. cinerea should be so prevalent on susceptible crops whenever conditions are suitable, the fact that the same can be said of diseases such as neck rot of onion caused by B. allii is less easily understood. This species is more or less confined to Allium spp. and most onion crops are grown from seed, so that unless the crop is grown on contaminated soil there should be very little or no introduction of the fungus with the crop. Moreover, the fungus is not particularly

evident on the growing crop even in wet seasons. It is, therefore, somewhat puzzling, at least to me, that neck rot is so often a major source of loss. I should be grateful for enlightenment on this problem.

Before dealing with the inactivation of Botrytis spp. with the more conventional types of fungicides, I should like to consider very briefly some other ways of controlling disease. There is, of course, the obvious one of not using contaminated or infected planting material. And because the fungi grow and sporulate freely on dead and moribund tissue, it might always be possible and with little extra effort to grow plants so that less of this type of tissue is produced. Colonization of any that is produced might be reduced by growing plants so that the moisture content of this tissue is kept at levels which preclude attack, or by encouraging a growth on it of saprophytes which will exclude the pathogen. There is a variety of ways in which this can be done in different crops, and there are examples in the literature where the control which has been obtained by cultural methods can be explained by one or other of these mechanisms. The potential advantages of these cultural and biological methods of control are, of course, that they need not add appreciably to the costs of production (they will not be adopted if they do) and they avoid the trouble of unpleasant and toxic residues in crops such as lettuce and strawberry. A disadvantage is that these methods are likely to be unreliable unless they are developed on a rational basis so that the mechanisms involved are pretty well understood. This will call for a much greater knowledge of the biology of Botrytis diseases than we now possess, and it will be a long time before this is available even if determined and systematic efforts are made to obtain it. Also, it is unlikely that biological and cultural methods will be fully effective even under favourable conditions. Because of this they would be best developed as adjuncts to the more conventional methods which use fungicides. This brings me conveniently, and with not too much time left, to my last topic, and the one which will be of greatest interest to most members of this audience - control with fungicides. It was my deliberate intention not to deal with this in any detail because I know that this is to be done by later speakers. There are, however, a few aspects I should like to mention briefly.

It is somewhat surprising that some fungicides, active against Botrytis spp. in vitro and which control foliage diseases caused by other pathogens, are relatively ineffective against Botrytis spp. in the field. I have in mind copper fungicides and in particular Bordeaux mixture, which do not seem to have been very successful in the control of Botrytis diseases. It might be, of course, that these fungicides are effective to start with but their phytotoxicity creates a new lot of damaged tissue on which residual fungus can multiply. The net result may be no control, or even an increase in the amount of disease. But this is unlikely to be the whole explanation. Another factor might be the inability of some fungicides to penetrate tissue which carries the pathogen internally. If such tissue were abundant, a fungicide which acts on the surface would only reduce the quantity of inocula temporarily. The inactivation, possibly by adsorption, of some fungicides by dead plant material containing the pathogen is also a possib-

ility and no doubt others will suggest themselves to you. Having said that I cannot understand why some fungicides do not control these diseases. I should now like to present the reverse side of the picture because I am also puzzled why certain others do control disease. I have in mind the chlorinated nitrobenzenes and related compounds which have been reasonably successful against Botrytis diseases. Until fairly recently this was their main use. Pentachloro-nitrobenzene, for long one of the most widely used members of the group, has not much effect on spore germination except to delay it slightly, and the fungus continues to grow on agar in the presence of high concentrations of vapour of the fungicide although at a reduced rate; 2,6-dichloro-4-nitroaniline is not much more effective. It is only the third of the extensively used of these compounds, 2,3,5,6-tetrachloronitro-benzene, which has any pronounced effect on spore germination and growth. Even this substance does not prevent spore germination. The one thing which can be said in favour of the three substances so far as in vitro tests are concerned is that each retards or prevents sporulation so long as the fungus is kept in sufficiently high concentrations. This effect is quickly lost when the fungicide is removed. From all this one might anticipate some control of disease in confined spaces in which quite high concentrations of vapour could be maintained, but in practice this cannot happen very often except perhaps in frames. In glasshouses and in the field the concentrations required for activity in vitro would only rarely be obtained, and it is difficult to see how these fungicides could be very effective as protectants, eradicants or antisporulants under these conditions. It is possible that they are effective not because they act directly upon the fungus but because they alter the host plant so that it becomes resistant. This would not be altogether surprising because in sufficiently high concentrations they can greatly affect the growth of plants. For example, penta -, and tetrachloronitrobenzene can retard the growth of lettuce without causing any other striking damage, and, of course, their ability to suppress sprouting of potato tubers in storage is well known and has some practical advantages. I have also wondered whether they are the sort of substances which would accumulate in the cuticle to concentrations which would be sufficient to reduce significantly the rate of growth of penetrating hyphae.

There remains another reason which makes the effectiveness of these fungicides rather surprising. In culture Botrytis spp. readily and fairly quickly produce strains which are almost completely resistant so far as vegetative growth is concerned. We know that resistant strains of B. allii retain their pathogenicity to onions and we are about to study the pathogenicity of other strains. All resistant strains retain their ability to sporulate, but most produce few or no spores in the presence of high concentrations of the fungicides. There are, however, some strains that do sporulate under these conditions. So far there is little evidence that resistant strains are of any practical importance. The place to look for these would be on crops where the use of chlorinated nitrobenzenes has not given so good a control as was expected. Mr. D. C. Gwynne and Mr. D. R. Humphreys Jones very kindly sent me lettuce and tomato plants from crops where this had happened but I found no evidence of resistance in

isolates of B. cinerea from this material. So we still wait for good evidence of resistance in the field to these as to other fungicides and in this respect at least we are more fortunate than our entomological colleagues.

This completes the detail of what I have time to say but it would be inappropriate in a conference of this sort to end on too optimistic a note. Speaking as I do from the sidelines, because I am not involved directly in the business of controlling diseases, it seems to me that at present there remain a number of diseases caused by Botrytis spp. which are not so readily or efficiently controlled with fungicides as are certain other diseases. It may be that we need better fungicides or ones which act differently from those now in use. But I also think it possible that we may ask too much of any fungicide to control some Botrytis diseases when conditions are particularly suitable for their development. The task set for the fungicide might be made considerably easier by the judicious use of cultural methods aimed at reducing the amount of dead tissue which is, or can be, colonized by the fungi. The function of the fungicide would then be to complete the job of control.

PROBLEMS IN THE CONTROL OF RASPBERRY AND STRAWBERRY GREY MOULD

by W. R. Jarvis
Scottish Horticultural Research Institute

During the last decade many fungicides have been used in attempts to control grey mould of raspberries and strawberries caused by the fungus Botrytis cinerea. In no case has even a moderately successful material been found which is not the subject of one or more serious objections when applied at the time of flowering and fruiting. Many fungicides, particularly when formulated as dusts, spoil the appearance of the fruit, one or two may be phytotoxic (e.g. dichlone and thiram (Cox and Winfree, 1957)), some may cause tainting, particularly in processed products (e.g. captan and thiram, (Marsh, Martin and Crang, 1955 and Crang and Clarke, 1961)), and all are subject to regulations regarding tolerated levels of residues.

A notable feature of the various field trials of prophylactic fungicides has been a marked lack of consistency in results. Only infrequently has a good level of disease control been attained, and results generally vary quite widely from year to year, even on the same plots, using the same fungicide and as nearly as possible the same rates and times of application with respect to crop development. More usually, the disease incidence is reduced by the best of the fungicides to only a third or even a half of that on the untreated control plots.

An examination of the pattern of crop development, of the biology of the parasite and of the techniques of fungicide application suggests some reasons for these variable results.

Both strawberry and raspberry fruits mature over a long period and, at any given time, flowers and fruit in all stages of development are present together. The normally recommended time for the first fungicide application is at petal fall, a rather poorly-defined term in view of the long period over which this occurs. It is, however, possible to discern a period of maximum petal fall, but at this stage some fruit is already ripening, while some buds are still very young and tightly closed. The recommended practice of two or three applications at 10-day intervals, inevitably contaminates considerable quantities of ripening fruit, but fails to protect the fruit developing from still closed buds.

Assuming that it is possible to tolerate a fungicide on maturing fruit, there are also almost insuperable physical and economic difficulties in maintaining a complete prophylactic cover on rapidly expanding fruit surfaces and Jarvis (1961) found that, in general, no better control was obtained by continuing fungicide applications throughout the picking season, than by stopping treatments about ten days before the first pick.

The problem in grey mould control are emphasised when the pattern of fruit infection is considered in relation to the biology of the parasite. While B. cinerea generally sporulates on organic debris in fruit plantations throughout the year and especially during the flowering and fruiting period, comparatively few infections can be ascribed to the text-book

mode of infection, i.e. by the germination of spores in water droplets on the surface of the fruit (Blackman and Welsford, 1916). Despite airborne spore concentrations of the order of 10^4/cu.m. frequently during the fruiting period (Jarvis, 1958), only about 1% of all fruit infections have been found to result directly from spore germination (Jarvis, 1961). Unfortunately it is this type of infection which is probably the most easily controlled by fungicides applied to the fruit surface. In a strawberry plantation (var. Talisman) in 1960, 64% of infections were found to result from the penetration by hyphae growing from adhering infested organic fragments, such as moribund petals, pieces of straw and weed debris, 18% from contact with rotting fruit, and 17% had been initiated from the calyx end. Because of the relatively cleaner conditions in raspberry plantations, there was virtually no infection of fruit from adhering organic debris. Petals are shed as in strawberries, but rarely adhere to the fruit for long enough to permit infection; the microclimate of the fruit and its surface contours do not favour petal adherence. Infection by contact with rotting raspberries and especially with rotting receptacles remaining after the berries were picked comprised 35% of infections, while 64% were initiated from the calyx end.

In strawberry plantations the problem of infection from infested organic debris is particularly important. In the variety Talisman, more than a quarter of a pound of such material consisting of dead leaves, petioled, stolons and straw, has been removed from a single crown in a plot which had been completely defoliated after the previous harvest and the debris raked off. Considerably more debris remains in non-defoliated crowns. Attempts to control the fungus in its over-wintering stage by applying a formulation based on phenyl mercury oxine, as a drench, in January and February have not yet been successful, and it is assumed that reinfestation of the debris occurred before the fruiting period. Colonisation of debris at any time of the year is rapid, and even straw newly laid as a mulch may become infested within 14-21 days. It seems most unlikely that any fungicide good enough to eradicate the fungus shortly before picking would be tolerable from the toxic hazard viewpoint. A small reduction in infection was obtained, however, in plots of the strawberry Talisman in which the straw mulch was previously treated with a waterproofing and fungicidal preparation of copper 8-quinolinate and also when black plastic sheeting or wet-strength paper impregnated with copper 8-quinolinate was substituted for straw. Some improvement in plantation hygiene by removing debris could be effected where post-harvest burning is practicable, but because of the climate, this has not yet been possible at Mylnefield.

The infection figures also emphasise incidentally the need to keep fruit picked frequently and, if possible, to remove infected berries.

Infection from the calyx end of the fruit is very important in both crops, and may result from one or both of two processes. Firstly, spores may be trapped between the sepals and the fruit surface in a film of water and germinate directly to infect the fruit, and secondly, infection of attached

moribund flower parts may occur, followed by infection of the fruit from an inoculum having a large saprophytic food base and therefore a greater inoculum potential, sensu Garrett (1956). A high inoculum potential also accounts for the striking success of infections from infested debris adhering to the fruit surface (Brooks, 1908). Even where spore germ tubes penetrate directly, their effect may be enhanced by the presence of solutes from moribund material (Brown, 1922). The relative importance of the two modes of calyx-end infection is unknown, and perhaps rather difficult to assess.

At all events, the airborne spore inoculum is of particular importance in initiating infestations at the calyx end and small scale experiments at Mylnefield with an antisporulant, hexachloro-2-propanol (Horsfall and Rich, 1960), alone and in combination with captan, to reduce the incidence of airborne spores have not been encouraging. In view of the considerable distance travelled by spores of B. cinerea it is unlikely that this approach will be effective, except possibly in isolated plantations.

Infection of strawberry flower parts occurs at a very early stage in development and they remain largely symptomless until the fruit begins to ripen. In 1961 isolations made almost a month before picking from scarcely-opened flower buds showed that 3.1% of carpels were already infested, 1.2% of the stamens and 2.0% of the sepals. These figures were approximately doubled in slightly frosted flowers. At the same time, 57.8% of detached petals adhering to other developing flowers and fruit were infested. A month later the infestation rates in newly fertilised flowers were: carpels 20.8%, stamens 17.0%, sepals 3.4% and undetached petals 3.1%. Corresponding figures for slightly-frosted flowers showing no grey mould symptoms were: carpels 26.0%, stamens 48.1% and sepals 5.3%.

For raspberry flowers and fruit the figures were even higher; 39.1% of all floral parts of unfrosted flowers and 49.5% of slightly frosted flowers, were found to be infested.

It has been recently reported from the U.S.A. (Powelson, 1960) that a latent mycelium is often present in apparently healthy green and ripening strawberries, particularly at the calyx end. This mycelium may remain quiescent in the fruit, even until after marketing, but is always potentially aggressive from the white fruit stage onwards. This finding has now been confirmed at Mylnefield and a latent mycelium has also been demonstrated in raspberries. The conditions inducing the change from quiescent to aggressive mycelium are still largely unknown; the physical and chemical changes in the ripening fruit are undoubtedly important, but there is reason to believe that these are not the only factors concerned. It seems probable, however, that the latent mycelium originates from infection of the floral parts at a very early stage in fruit development and the problem of controlling calyx-end infection appears therefore to be largely one of controlling flower infection from the closed bud stage onwards.

It is evident that the normally recommended time of the first fungicide application is probably too late for prophylaxis, and that the internal

flower parts should be protected as soon as they are exposed. The difficulties of achieving this when flowers do not develop simultaneously have already been mentioned and there are also technical difficulties in adequately protecting partially covered flower parts in opening buds.

The fungicides commonly used for the control of grey mould have been shown to be quite effective against the fungus in other crops, e.g. in glasshouse lettuce, but are frequently disappointing in strawberry and raspberry crops. The application of fungicides to mature fruit plantations often necessitates light equipment and low volumes, and it seems likely that these methods do not ensure adequate coverage of all the flower parts. Recent work with strawberries in Germany (Müller, 1961a, 1961b) suggests that very high volumes and high fungicide rates combined with an improved technique, might bring about considerable improvement in control. Thus thiram applied at the rate of 4.8 kg/ha (4.4 lb/acre) (the normal rate being 2.0 kg/ha) in 2,400 l/ha (218 gall/acre) reduced infection from 52.1% to 4.8%. The spray was directed well into the plants from the top and both sides by a triple arrangement of jets, the emphasis being on obtaining a thorough drench. Similar recommendations for strawberry grey mould control in the U.K. have been made by Moore (1961). Captan and dichloronitroaniline applied at Mylnefield in 1961 at rates comparable to those used by Müller, but without the special arrangement of jets, did not give significantly better control than the normal low volumes in strawberries, but in raspberries where a captan drench was directed straight into the young flowers and fruit, infection was reduced from 14.2% to 8.6%. This reduction, however, by Muller's standard is still poor.

It might be supposed that systemic fungicides would give some protection to the flowers and fruit without the necessity of drenching, but many attempts to control the disease with applications of griseofulvin to the flowers and foliage have not been encouraging in either crop.

It is evident that the overall problem of grey mould control in soft fruit may be subdivided into a number of problems, each of which requires a separate approach in considering both fungicide and technique.

General plantation hygiene is of great importance in reducing the potential inoculum in the form of overwintering mycelium and sclerotia in organic debris, which also supports the actively saprophytic and sporulating stages of the fungus. In plantation hygiene may be included weed control, so that at once both an important incoulum source and, particularly in the case of strawberries, an environment conducive to increased sporulation and infection is removed. Attention to conventional methods of plantation management thus can do much to increase the efficiency of fungicides. It seems most likely that when presently available fungicides are carefully selected with respect to the biology of the crop and of the pathogen and of the two in combination, and are applied by improved techniques, at the biologically correct times, control of the disease could be achieved economically and without detriment to the quality of the crops.

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OBSERVATIONS ON THE CONTROL OF BOTRYTIS IN ANEMONES

by A. Elizabeth Jeff
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Summary

Botrytis cinerea will attack Anemones at times during the season when the plant has suffered damage during adverse weather conditions in autumn and winter, or when the plants have been previously weakened by Peronospora infection during summer and autumn and when the weather favours the spread of the fungus.

In trials over a period of four years spraying with zineb, captan and thiram every fourteen days from September to March showed good but variable results. In two seasons captan gave the best results in keeping plots free of Botrytis during the winter months following bad weather, but in the other two years zineb proved the better fungicide. In this case a high incidence of Peronospora occurred in summer and autumn and zineb had given more protection from this disease increasing the plants resistance to frosts with a consequent lower incidence of Botrytis infection.

Tecnazene dust, streptomycin compounds were found of little value, and all compounds containing copper caused severe plant damage.

Dicloran appears promising, the spray formulation being better than the dust.

Introduction

The season of growth of the Anemone plant in the South West is from June when the corms are planted, through the autumn and winter given good weather conditions until March or April. Flowers are produced more or less continuously from September onwards. Thus the Anemone, growing as it does through the winter months is often subject to severe gales and the occasional frost interspersed with periods of mild humid weather. It is at these times when attack by Botrytis can be widespread and serious. Another factor which must be taken into consideration is the amount of Peronospora ficariae (Anemone Downy Mildew) present. This disease spreads in humid conditions in summer and autumn, considerably reducing the plant's resistance to bad weather and consequent Botrytis attack. Being a systemic fungus which slowly reduces the vigour of a well grown plant after secondary infection it is not always possible to tell in the field whether P.ficariae is present or not. Neither the summer or winter sporing stages can be positively identified without the use of a hand lens and in many cases these spores will not be present although the plant is infected.

Treatments

Fungicides which have been in constant use since the trials commenced in 1956 are:-

Zineb at 2 lb of a 65% material in 100 galls water/acre.
Captan at 2 lb of a 50% material in 100 galls water/acre.
Thiram at 2 lb in 100 galls water/acre.

In each season spraying was commenced at the end of August and continued at fourteen day intervals throughout the season weather permitting.

Results

The results of the trials are assessed on the number and quality of flowers and the time at which they are cropped. Visual observations on the general appearance of the plants were made, but correct estimations of the amounts of Botrytis and Peronospora present could not be made without microscopic examination of individual plants facilities for which were not available.

The tables below are the results of the trials of four seasons set out to show the numbers of good quality flowers cropped in successive four week periods from plots of 160 corms. The total numbers in general vary somewhat from year to year but this is only due to differences in actual growth rates in different seasons.

Numbers of good quality blooms cropped in successive 4 week periods. Plots of 160 corms

1956 - 1957

4 weeks ending	Zineb	Captan	Thiram	Control
13 October	10.2	8.9	11.8	4.4
10 November	30.9	26.5	30.2	24.4
3 December	19.5	17.5	16.9	13.3
5 January	6.2	6.3	6.9	4.4
2 February	2.4	5.5	4.4	2.7
2 March	9.5	8.9	5.5	0.4
30 March	55.3	37.3	24.4	13.3
27 April	26.5	20.0	15.5	7.5
4 May	54.0	37.8	39.1	16.4
Totals	214.5	168.7	154.7	86.8

1957 - 1958

4 weeks ending	Zineb	Captan	Thiram	Control
29 September	3.8	3.5	3.4	3.1
26 October	22.9	31.1	26.7	26.2
23 November	43.4	44.3	49.3	38.7
21 December	24.9	28.7	24.4	26.7
18 January	53.3	68.9	52.3	60.8
15 February	38.4	63.1	40.9	19.5
15 March	53.8	114.7	62.9	14.7
12 April	9.3	20.0	6.9	0.4
Totals	249.8	374.3	266.8	190.1

1958 - 1959

4 weeks ending	Zineb	Captan	Thiram	Control
20 September	34.0	37.5	18.0	25.3
18 October	85.0	95.5	62.8	80.0
15 November	90.8	107.0	72.0	91.8
13 December	24.5	36.3	30.8	32.0
10 January	20.3	30.0	17.3	24.0
7 February	10.3	20.5	10.3	10.0
7 March	18.0	36.5	28.8	21.5
21 March	7.3	27.8	14.3	10.3
Totals	290.2	391.1	254.3	294.9

1960 - 1961

4 weeks ending	Zineb	Captan	Thiram	Control
27 August	0.2	-	-	-
24 September	23.7	17.8	15.7	25.7
22 October	78.5	46.3	55.3	77.2
19 November	66.5	55.3	49.7	64.5
17 December	23.5	25.7	21.0	20.3
14 January	5.7	8.7	4.5	4.2
11 February	7.3	9.8	4.3	1.7
11 March	54.2	39.2	25.7	3.7
8 April	56.7	32.2	23.0	3.2
6 May	8.3	3.7	4.5	-
Totals	324.6	238.5	203.7	200.5

The figures underlined are those of particular interest as they indicate the greatest effects on yield due to the spraying treatments.

The table for 1959 - 1960 has been omitted, this season being a particularly bad one for the growth of Anemones and spraying with any fungicide was of little value.

1956 - 1957 Season

Spraying up to the end of December appeared to have no effects on plant health and gave no significant differences in yield. At the end of December a series of sharp frosts occurred the last of which was on 1 January. The ninth application of the spray treatments was applied on the following day under ideal conditions. The following day the weather changed completely with minimum night temperatures of 44° F. to 53° F and a relative humidity of 93% to 100%, ideal for the spread of Botrytis on frost damaged foliage. From this point differences between the treatments became apparent. Botrytis spread rapidly on the control plot and to a certain extent on the captan and thiram plots but zineb gave a clear indication of having maintained plant health to a fairly high degree. The effect on crop yields was not immediate but in March the number of flowers cropped was well above the control and better than captan and thiram.

1957 - 1958 Season

Here again beneficial results were obtained during the weeks following the new year but the results showed a surprising reversal in the effectiveness of zineb and captan, the latter giving by far the best results. General observations of the plots showed no reason for this as growth of the

Anemones had been reasonably uniform in both seasons. The answer could only be found by repeating the trials. One thing however, was confirmed, the necessity of spraying during the winter months after bad weather.

1958 - 1959 Season

The results of this season were similar to those of 1957 - 1958 with captan again giving the best results. The differences did not show until nearer the end of January when frost had damaged plants. Zineb was again disappointing in fact it appeared less effective than thiram.

1960 - 1961 Season

Rainfall was frequent during summer and autumn which made for good regular growth of plants and also for rapid secondary spread of Peronospora. Visual effects of all sprays were showing by mid-November there being more Botrytis infection on the control plot. A series of ground frosts occurring in mid-December followed by mild humid weather, similar conditions to those in 1956 - 1957, caused further differences to show between the treatments. In this case zineb repeated the success it had given in the first year's trials, with captan not nearly so effective. Attention was then drawn to other similarities between these two seasons. It was known that there was a high incidence of Peronospora infection in the summer and autumn of 1960, and research into records of another trial dealing specifically with Peronospora also showed that the disease had spread rapidly in 1956, but not to such an extent in the other two years. The probable explanation of the success of zineb in these circumstances is that this fungicide had been more specific in controlling Peronospora, thus giving the plants more resistance to adverse weather conditions, particularly frost.

Other fungicides

Early trials include a number of other fungicides which for various reasons were discontinued in further trials. Those which must be mentioned as causing severe damage by leaf scorch are all compounds containing copper in whatever form. This may be partly due to interaction with the salt in the atmosphere of the South West maritime climate, a point which must be considered whenever damage of this kind occurs. Other fungicides of little effect were tecnazine dust and some antibiotic sprays containing streptomycin. In general spray formulations of fungicides are far more effective than dust formulations of the same compounds, captan must be mentioned in this respect.

Of the newer materials tried recently the spray form of dicloran has given promising results; almost comparable with captan in a good captan year. Trials at Ellbridge sub-station have also shown maneb as promising.

Discussion

The varied results given by different fungicides indicate that control of Botrytis in Anemones is not by any means straight forward. The first conclusion drawn in the early years was that spraying was necessary

after mechanical damage due mainly to weather conditions. It did not at that time appear necessary to spray against Botrytis during the summer and autumn, and captan applied at the correct time would give the best results. But there were these two seasons when captan had failed which made this procedure questionable. It seemed that there was some factor involved which nullified the effect of a direct Botrytis controlling spray. From the results obtained with zineb it then became fairly clear that severe infection by Peronospora had an effect on plant stamina not always obvious during summer and autumn. If this disease was not controlled the chances of efficient control of Botrytis later in the season were considerably reduced. These trials have led to a revision of the general spraying programme at Rosewarne. Spraying with zineb is carried out regularly during August and September until the aerial spread of Peronospora is no longer apparent. An occasional captan spray is applied at this time if it is thought necessary and during the autumn and winter months this material is used regularly in place of zineb.

THE FUNGITOXIC EFFECTS OF DICLORAN ON BOTRYTIS CINEREA

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Summary

Dicloran (2, 6-dichloro-4-nitroaniline) is fungistatic to the mycelium and spores of Botrytis cinerea. It delays germination and causes a severe check to hyphal growth. In certain media, dicloran causes distortion and bursting of the germ tubes. The nucleic acid level of B. cinerea mycelium increases in the presence of dicloran; the most marked increase occurs at low dosages (1 p.p.m.) when growth is checked without morphological abnormality. It is suggested that dicloran is a structurally non-specific toxicant exerting its effect by disorganising cell growth and division.

Introduction

For many years, Botrytis cinerea, the fungus causing grey mould on glasshouse lettuce crops has been controlled by the use of chlorinated nitrobenzene compounds. More recently, however, 2, 6-dichloro-4-nitroaniline (dicloran) has also found commercial application for the control of this disease.

Laboratory and glasshouse experiments have indicated that dicloran and the chlorinated nitrobenzenes possess a number of common features and that they are active against a similar range of fungi. Thus, experiments on the mode of action of dicloran against B. cinerea have been designed in the light of results available from earlier work with the chlorinated nitrobenzenes.

Clark, Hams, Higgons and Stevenson (1960) have shown that, although dicloran has negligible activity in the slide germination test (American Phytopathological Society, 1943), its high toxicity to B. cinerea can be demonstrated by an agar impregnation test (Brook, 1952). The activity of tecnazine against this fungus may also be demonstrated by the impregnation test but tecnazine resembles dicloran in being ineffective in preventing spore germination on glass slides. Differential activity against spore germination and hyphal growth of Monilia fructicola has been described for a range of compounds by Horsfall and Rich (1953) and it has been suggested (Horsfall, 1956) that since spore germination (unlike hyphal growth) is independent of nuclear division, these compounds may exert their effects by checking mitosis. Horsfall attributes the inhibition of sporulation by the chlorinated nitrobenzenes to their effect on mitosis and some support for this suggestion is provided by the work of Carey and McDonough (1943) who showed that p-dichlorobenzene causes polyploidy in onion root cells.

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A series of laboratory experiments were therefore undertaken to compare the general effects of dicloran and tecnazine on spore germination and mycelial development in B. cinerea. An investigation was also begun into the effects of sub-lethal doses of dicloran on nucleic acid synthesis in the cells of this organism.

Materials and Methods

Fungicides

Phenylmercuric chloride (PMC) and phenylmercuric nitrate were used as the finely ground technical materials. These compounds were added to the medium in 0.5 ml. sterile distilled water, the concentration of the suspension being adjusted to give the correct final concentration upon dilution.

2,6-dichloro-4-nitroaniline and 2,3,5,6-tetrachloronitrobenzene were obtained by re-crystallisation of the technical material. These compounds were added to the medium in 0.5 ml. acetone, the concentration of the acetone solution being adjusted to give the correct final concentration upon dilution.

Fungus

A sporulating strain of Botrytis cinerea was used for all the experimental work. Cultures were isolated from natural infections on lettuce plants. Stock cultures were maintained on potato extract agar (200 mg/litre decoction). Spore suspensions were prepared by rinsing the surface of the cultures with sterile distilled water. For most purposes the inoculum was adjusted to give a final concentration of 50,000 spores per ml. Where mycelium was used as inoculum, uniform discs (7.5 mm. diam.) of malt agar bearing non-sporing mycelium from a 3-day-old culture were cut from a petri dish and placed in the culture medium.

Media

Malt extract, prepared by diluting malt extract with water to give a 2% solution.

Czapek, prepared as follows: sucrose (3.0%), sodium nitrate (0.2%), potassium chloride (0.05%), potassium phosphate (0.1%), magnesium sulphate (0.05%), ferrous sulphate (0.001%). Normally this medium was maintained at pH 4.0 using McIlvaine citrate-phosphate buffer, diluted with two parts of water.

Dextrose citrate, prepared as a solution containing 0.1% dextrose and 0.001% sodium citrate.

The above media were sterilised by autoclaving for 20 minutes at 20 lb./sq. in. Agar (2%) was used to solidify media where necessary.

Culture Methods

Cultures were grown in liquid media using bottles (vol. 350 ml.) outside dimensions 14.5 x 4.5 cm. placed horizontally with the largest side downwards. Petri dishes were used for experiments using solid media. Slide cultures were prepared by the method described by Knaysi (1957) and spore germination tests were carried out by the technique recommended by the American Phytopathological Society (1943). In all cases the organism was incubated at 23° C.

Assessment Methods

Mycelium grown in liquid cultures was separated from the medium, washed with distilled water and then dried to constant weight at 50° C. For more critical work the washed mycelium was freeze-dried before weighing. Linear growth on solid media was measured along two diameters of the dish at right-angles. In all experiments at least five replicates were used for each treatment. Germtube development in slide cultures was estimated by a grading system based on the number of septa evident at the different stages of development.

Dosage response data was analysed by the probit method and toxicity was expressed as the dosage required to reduce germination or mycelial growth by 50% (E.D.50 value). Saturation response data were treated in a similar way to obtain the chemical potential or R.S.50 value for different fungicides (Byrde and Woodcock, 1959). Saturated solutions were prepared by adding an excess of the finely-ground fungicide to sterilised medium and placing the flasks on a mechanical shaker at room temperature. The media were allowed to stand overnight and the clear supernatant was decanted off into sterile flasks. The saturated solution was then added to culture bottles containing the appropriate volume of normal medium to give a series of dilutions. Normally a dilution interval of one half was used, starting from an initial relative saturation of 0.25.

Determination of nucleic acids

The preliminary extractions of acid-soluble-phosphorus and lipid-phosphorus were carried out by the methods of Smillie and Krotkov (1960). The residue from these preliminary extractions was then dried, powdered and extracted three times with 2 ml. of 0.5 N perchloric acid at 70° C. Each extraction was continued for 30 minutes, the supernatants being combined. The total nucleic acid content of the sample was determined by measuring the extinction of the extract at 260 mμ.

Replicate comparisons were made on freeze-dried mycelium of B. cinerea to establish the reliability of the technique. Repeated determinations made on the same samples of mycelium indicated that an error of not more than 10% could be expected in the values for total nucleic acid obtained by this method.

Experimental

The first series of experiments were carried out to examine the general effects of a range of concentrations of dicloran on spore germination and mycelial growth in a variety of solid and liquid media. These were followed by experiments designed to study the specificity and reversibility of the toxic action of dicloran.

Solid media

On malt extract agar, a concentration of 1.07 p.p.m. dicloran was required to suppress mycelial growth of B. cinerea by 50%. When Czapek agar was used to culture the fungus, 1.45 p.p.m. dicloran was needed to obtain the same degree of inhibition.

Spore germination and subsequent mycelial development of B. cinerea on agar impregnated with varying concentrations of dicloran and tecnazine was examined under normal and phase-contrast microscopes. Slide-cultures were set up in which the agar was impregnated with 5, 25, 100 or 500 p.p.m. of either dicloran or tecnazine. Untreated agar was also inoculated with spores and served as control.

Even at the highest concentrations of dicloran and tecnazine the rate of germination and subsequent hyphal growth was only reduced to about a third of the rate recorded on untreated agar and eventually sporing colonies were produced. No morphological effects were evident under these conditions, the frequencies of septa and lateral branches being no different from the control. No spore germination was recorded in similar slide-cultures impregnated with 10 p.p.m. PMC.

In a further experiment, slide cultures were prepared from a small portion of the same impregnated agar as that used for plate cultures. The slide and plate cultures were then inoculated with spores from the same culture and, whenever observations were made on the slide cultures, discs of agar bearing the spores and developing colonies were taken from the plates and also examined. In a comparison of the effects of 10 p.p.m. PMC and 10 p.p.m. dicloran on germination of spores of B. cinerea in the two types of culture, no germination was observed within 8 days in the presence of PMC, in either the plate or slide cultures. Furthermore, no germination occurred during this period in plate cultures impregnated with dicloran although slow growth occurred in the slide cultures and the mycelium began to sporulate by the fifth day after inoculation.

Liquid media

The toxicity of dicloran to mycelium of B. cinerea in liquid culture was examined in accordance with Ferguson's Principle (Ferguson, 1939), where the inadequacy of expressing toxicity in terms of applied concentration is overcome by using the chemical potential (or thermodynamic activity) of the fungitoxic compound. The chemical potential of toxicants, applied in solution, may be expressed as S_t/S_o where S_t is the molecular concentration of the toxic solution and S_o its solubility in mol./litre. Thus the concentration required for 50% inhibition of mycelial growth was express-

ed as relative saturation (R.S.50) rather than dosage (E.D.50). Parallel tests were carried out with PMC. The R.S.50 value of dicloran against B. cinerea in Czapek medium was found to be 0.21; that of PMC was 0.0004 under the same conditions.

Spores of B. cinerea germinated within a few hours when added in droplets of nutrient solution on to clean glass slides. In the presence of dicloran, no growth check was visible until the fungistatic concentration exceeded 8 p.p.m. As with solid media in slide cultures, concentrations of dicloran up to 500 p.p.m. merely caused a retardation in growth rate and no gross changes in morphology occurred. Reducing the concentration of spores in each droplet from 100,000/ml. to 80/ml. did not significantly increase the fungitoxicity of a given quantity of dicloran.

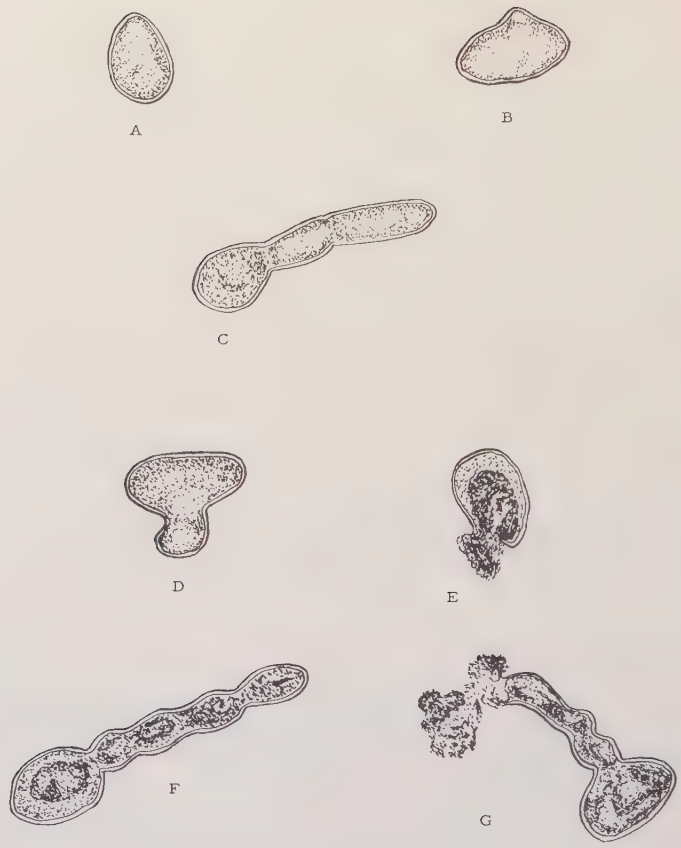
Dicloran produced a marked effect on hyphal growth when introduced into flask or bottle cultures. With most natural isolates of B. cinerea, germination was almost completely inhibited at 5 p.p.m. or above and at lower concentrations very severe distortion of the germ tubes was caused. Thus spores produced short germ tubes which became constricted at intervals along their lengths and often swollen at their tips. Frequently the germ tube would burst allowing the protoplast to stream out from the ruptured tip. The effects are illustrated in Fig. 1. The optimum concentration for the more severe morphological effects lay between 1 and 5 p.p.m. but also occurred at slightly higher concentrations with some isolates. Below a concentration of 1 p.p.m. the germ tubes showed less tendency to burst and only slight malformation. The growth rate was, however, still checked under these conditions.

When Czapek medium was replaced by dextrose citrate medium for germinating spores in bottle cultures, no bursting of the hyphae occurred in the presence of dicloran at 25, 5 or 1 p.p.m. Germination was very slow in this medium but, after 5 days, germ tubes were produced in cultures containing 5 and 1 p.p.m. dicloran. The germ tubes showed none of the abnormalities associated with Czapek medium. On the other hand, in similar experiments using a liquid 2% malt extract medium, deformed hyphae and burst germ tubes were again produced in the presence of 10 and 2 p.p.m. dicloran.

Reversibility of Toxicity

The term "fungicidal" is normally applied to a fungitoxic compound which penetrates to the site of action within the protoplast where it causes irreversible damage to the metabolism of the cell. On the other hand a "fungistat" effects its toxicity only while an adequate concentration of the substance is maintained at the site of action and thus fungistatic effects are normally reversible, once the fungitoxic concentration is reduced.

In a preliminary experiment to examine this point, discs of mycelium were grown in liquid Czapek medium containing 10 p.p.m. of phenylmercuric nitrate (PMN), tecnazine or dicloran. A further set of discs were grown in media containing no added fungicide. No growth occurred in any of the treated cultures but, after 10 days, the discs were taken from the fungitoxic solutions, washed in sterile water and then transferred



A - C. Control series in normal Czapek medium.
D - G. Treated series in Czapek medium containing 5 p.p.m. dicloran. A. B. cinerea spore before germination.
B. 6 hours after germination. C. 18 hours after germination.
D. 18 hours after germination in treated medium.
E, F and G. 48 hours after germination in treated medium showing deformed and burst germ tubes exuding contents from hyphal tip (x 2,500).

Fig. 1

aseptically to fresh culture bottles containing no added fungicide and incubated for a further 10 days. At the end of this period discs receiving pre-treatment with either tecnazine or dicloran were growing normally and at the same rate as the mycelium in bottles inoculated with discs receiving no pre-treatment. However, discs receiving pre-treatment with PMN showed no signs of growth and had evidently been killed by this fungicide.

A quantitative experiment was then carried out to determine whether full recovery occurred or whether dicloran left a residual effect. Discs of mycelium were held in liquid cultures containing 50 p.p.m. dicloran for periods varying from 10 - 70 days. After the given period, the discs were washed and transferred to culture flasks containing no added fungicides and incubated. 10 days later the dry weight of mycelium was determined and compared with the dry weight of mycelium produced by discs of the same culture strain which had received no pre-treatment with dicloran. The results (expressed in Table 1 as the percentage reduction in dry weight) suggest that recovery is virtually complete when the fungus is transferred within 2 or 3 weeks to media containing no added fungicide. However, when held in the presence of the toxicant for over a month, recovery may be slightly delayed.

Table 1 The effect of period of inhibition by dicloran on rate of recovery of mycelial discs of B. cinerea

Period of inhibition (days)	10	20	30	50	70
Reduction in dry weight (percentage)	0	5	10	26	35

The fungistatic nature of the toxicity of dicloran was again demonstrated by incubating spores of B. cinerea in dextrose-citrate liquid cultures in the presence of 25, 5 and 1 p.p.m. dicloran. After 15 minues, 18 hours and 43 hours, samples of the spores were centrifuged down, washed in sterile distilled water and then resuspended in medium containing no added fungicide. In all instances germination took place within 24 hours of transfer and after pre-treatment at 5 and 1 p.p.m., the growth rate in untreated media was normal. Some check in the growth rate was, however, noted during the first 24 hours following pre-treatment for 18 or 43 hours with 25 p.p.m. dicloran.

The effects of dicloran on nucleic acid synthesis

The effects of decreasing concentrations of dicloran on the nucleic acid level in mycelium of B. cinerea were examined. Spores were germinated in 5, 2.5 and 1.25 p.p.m. of dicloran and incubated in liquid Czapek medium contained in culture bottles for 48 hours. Two sets of untreated cultures were grown under the same conditions and harvested after 24 and 48 hours. Slide preparations were made from small samples of the different cultures at harvest. Spores germinating in the presence of 5 p.p.m. dicloran showed bursting at an early stage and only about half

the spores successfully produced germ tubes, most of which were severely stunted and deformed. In 2.5 p.p.m. dicloran, germ tubes were again malformed but the majority of the spores had germinated without bursting. In 1.25 p.p.m. dicloran the germ tubes were only slightly distorted but were less than half the length of the germ tubes of the 24-hour control cultures. Table 2 shows the total nucleic acid in the treated cultures expressed as a percentage of the freeze-dried weight of the mycelium. The total nucleic acid in untreated mycelium at a similar stage (24 hours) of growth and after a similar period of time (48 hours) to the treated cultures is also given.

Table 2 Effect of different concentrations of dicloran on total nucleic acid in B. cinerea mycelium

Age of culture (hours)	Treatment	Total nucleic acid (percentage of dry weight)
24	Untreated	1.48
48	Untreated	1.08
48	Dicloran 5.0 p.p.m.	1.34
48	Dicloran 2.5 p.p.m.	2.01
48	Dicloran 1.25 p.p.m.	3.45

The results given above suggest that total nucleic acid in B. cinerea cells is considerably increased by the presence of sub-lethal amounts of dicloran. The highest increase is associated with a relatively low dicloran concentration of 1.25 p.p.m. Presumably this concentration permits RNA synthesis but slows up cell growth. At higher concentrations more drastic mechanical effects are produced, so that any trend towards increasing nucleic acid content is offset by the larger proportion of spores which fail to produce intact germ tubes and therefore cease to synthesise nucleic acids.

The total nucleic acid level was shown to vary in untreated cultures of increasing age and, to avoid variations which might be due to age rather than to the presence of dicloran, the compound was added 6 hours after the start of the experiment to a proportion of the culture bottles, when germ tubes were just beginning to emerge from the spores. The cultures were harvested after 48 and 96 hours. The results are given in Table 3.

Table 3 Effect of dicloran (added after 6 hours) on total nucleic acid in B. cinerea mycelium

Age of culture (hours)	Total Nucleic Acid (% of dry weight)	
	Untreated	Dicloran (2.5 p.p.m.)
48	1.15	2.95
96	1.62	3.21

Initial estimates of the nucleic acid level in mycelium harvested after 24 hours failed to show significant differences between the dicloran-treated and untreated cultures. However, by 48 hours the total nucleic acid level in the dicloran-treated cultures had more than doubled, and by 96 hours the total nucleic acid in the treated cultures was considerably in excess of that of the untreated cultures at any stage of their growth.

Discussion

The spore germination experiments illustrate a marked reduction in the activity of dicloran when tested on microscope slides rather than in plate or bottle cultures. The same discrepancies were recorded in both liquid and solid media. It is evident, however, from the high R.S.50 value that a relatively high concentration of dicloran must be maintained in the fungal cell to effect maximum activity and it is possible that availability of the toxicant may have been limiting the activity of dicloran on glass slides. These results illustrate the limitations of the slide germination tests in the evaluation of certain groups of funtitoxic compounds.

The results of the experiments described above indicate that dicloran is fungistatic towards mycelium and spores of B. cinerea. In this respect it resembles tecnazine and differs markedly from PMN which is a true fungicide causing irreversible toxicity. However, there is evidence (Table 1) that prolonged exposure to dicloran in liquid culture causes some residual inhibition after the fungistat has been removed from the medium. This may simply reflect the occurrence of a longer lag-phase where the period of inhibition has been relatively long, since the data given in Table 1 are based on the dry weight of mycelium produced in the 10-day period following transfer.

The reversibility of the toxicity of dicloran, together with its relatively high thermodynamic activity suggest that its mode of action is structurally non-specific. It should be noted, however, that an R S.50 value over 0.1 may, in some cases, be due to the detoxification of the fungicide and is therefore not an invariable guide to non-specific toxicity.

Although dicloran does not prevent the germination of B. cinerea spores, it does delay the appearance of the germ tubes and causes a severe check to hyphal growth. Under certain conditions it may also cause distortion and bursting of the germ tubes. Other fungitoxic substances, such as α-naphthylamine and camphor (Bauch, 1941) and actidione (Wallen et al. 1950) produce similar, abnormal fungal cells while Priest (1960) has recently reported that the dichloronitrobenzenes produce severely misshapen hyphae in B. allii. Since differential activity towards spore germination and hyphal growth may be indicative of a toxic mechanism preventing cell division, and hyphal distortions have been found in association with mitotic aberrations caused by a range of other fungicides (Horsfall, 1956), it is probable that dicloran disorganises the processes involving cell division.

It is evident from Tables 2 and 3 that this disorganisation results in a considerable increase in the nucleic acid level of the cells. From other

data, not quoted here, it was apparent that about 80 - 85% of the total nucleic acid content of the extracts represents RNA and thus, there must be a significant increase in the RNA of the treated cells. Since the nucleic acids are responsible for cell reproduction and protein synthesis the increase recorded here, supports the view that dicloran is effecting its toxicity by disorganising cell growth and division.

It is not yet clear how the increase in RNA is caused but, in view of the relative ease with which aniline dyes form complexes with polynucleotides, it is possible that dicloran may form a weakly bound complex with the cytoplasmic nucleic acid and thus block protein synthesis. The reversible nature of dye-polynucleotide complexes (Steiner and Beers, 1958) would be in keeping with a non-specific, fungistatic mode of action.

Acknowledgements

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CONTROL OF FUNGI AND NEMATODES BY SOIL STERILANTS

Chairman: L. Broadbent
Glasshouse Crops Research Institute

EXPERIMENTS WITH SOIL STERILANTS, WITH PARTICULAR REFERENCE TO SODIUM N-METHYLDITHIOCARBAMATE (METHAM - NA)

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I. Introduction

This report deals only with a group of total soil sterilants for the action of which the biocidal properties of methylisothiocyanate have been taken as hypothesis. Methylisothiocyanate (MITC) is either introduced as such into the soil or is formed in the soil by chemical break-down from Metham-Na or 3,5-dimethyl-tetrahydro-1,3-5, 2-H-thiadiazin-2-thione (DMTT).

The three afore-mentioned products are officially approved in Germany by the Federal Biological Institute for Agriculture and Forestry for the control of harmful soil organisms including nematodes. They are used for the control of nematodes, soil fungi, weeds and arthropods living in the soil, but however do not act against soil bacteria. In our country these products are used mainly in horticulture, nurseries, but also in specific agricultural crops as for example in hops and in seed potatoes. The relatively high costs for the product and for the treatment do not allow a broader use in general agriculture.

The substances may be used under glass as well as in the open.

Several reports on the testing and use of these substances have been published in Germany. The following findings are based on these publications and on further unpublished results of our investigations. This report will deal with experimental methods, the mechanism of activity and the practical use of the products mentioned earlier.

II. Experimental Methods

We deal mainly with the fungicidal and herbicidal testing of soil sterilisers, whereas nematicide tests are not carried out in our laboratories. On such pests, however, Pieroh et al. (1959) have reported.

For the determination of the fungicidal potency of a soil sterilant, the use of 'Fungus Discs' which can be variously modified, have proved very suitable. The test fungus is grown through a rather thick filter-paper disc which is soaked with a substrate containing the necessary nutrients. Thereafter the fungus is introduced in this vegetative form to the soil, and we try later to reisolate the fungus from the 'discs'.

This method is described in the paper by Linden and Schicke (1957). The conditions under which the soil sterilant and the test disc are brought together are governed by the nature of the problem posed. If screening is concerned, the soil sterilant is admixed evenly to the soil and several chains of 'Test Discs' threaded on lines, are then put into the soil. The complete chains are then removed from the soil at intervals of 1, 2, 3 and 4 days and are brought to agar slants. The tests can, however, also be carried out by introducing the discs strata-wise into a soil column. By applying the product to the soil surface or to the base of the column, the spontaneous penetration into the soil can be established after removal of the 'Test Discs'. In this way it is possible by model experiments to ascertain the influence of varying soil types, of soil humidity, temperatures, treatment periods, etc. and on soil penetration.

The same method can also be used to examine the fungicidal efficacy of a soil sterilisation in field tests. It goes without saying that some modifications are, however, necessary. Domsch and Schicke (1960) have used foam rubber blocks soaked with nutrient solution which after formation of fungus growth - were placed in open-weave plastic bags and buried in the treated soil. By fixing a cord to each bag with a wooden handle above the ground, they could easily be located for subsequent removal, when they were packed in sterile glass tubes. Re-isolation of the fungus could then be carried out in the laboratory.

As test fungi for this method Fusarium culmorum, Thielaviopsis basicola, Verticillium dahliae, Rhizoctonia solani, Sclerotium rolfsi, Sclerotinia sclerotiorum and Pythium spp. can be used. As there are always bacteria adhering to the buried discs, it is appropriate to add an antibiotic to the nutrient substrate. In order to find out the actual mortality of the test fungus in the 'Test Discs' it is necessary to observe the re-isolation over a period of 15 days because seriously-affected fungi may only germinate after a long period. Naturally, this method also has its limitations. Even by adding several antibiotics, an elimination of all soil bacteria cannot always be achieved, particularly in soils rich in organisms and with long exposure times. In these cases, we have to reckon with contamination, in particular when using slowly growing test fungi. If very resistant test organisms are involved it can happen that when low concentrations of a substance are used, the test fungus is inhibited by some sort of infection. With medium concentrations it grows quite well however, and it is only by the use of high concentrations that a kill can be obtained without these undetermined 'infections'.

Foam rubber has hitherto, been found to be the best substrate carrier. One must, however, observe that the soil sterilants can penetrate more easily into this porous substance than into normal soil and thus the effective 'minimum concentration' of a soil sterilant can be much lower than in soils.

Finally, there can of course be objections regarding the use of infective fungi in these soil tests. In such cases the test fungi carriers should

be 'planted' conveniently for the subsequent excavation and removal of the surrounding soil, should the substance tested fail to achieve control. The herbicidal effect was measured by the introduction of seeds, which can also be placed in layers. We used cress (Lepidum sativum) for this purpose.

The 'waiting period' was checked with different plants.

III Mechanism of Action

We can distinguish between a physical and a chemical mechanism of action.

1. The physical mechanism of action

The first type of mechanism provides that the applied product reaches the point in the soil at which it must act in order to obtain a sufficient soil sterilisation. In this respect, we have been mainly concerned to find generally acceptable conclusions regarding the efficacy of a product, based on the method of application and the type of soil. For good effect, the prerequisite of a soil sterilant is an even penetration of the soil in sufficient concentration. This penetration can be achieved to some degree by the product itself, and assisted physically by the user. Only products with a high vapour pressure are enabled to penetrate spontaneously. The lower the vapour pressure, the more is mechanical distribution in the soil required. The products discussed in this paper possess a more or less high vapour pressure. For this reason Metham-Na and MITC can be injected into the soil or be applied in a trench or plough furrow without the necessity of subsequent mechanical distribution through the whole area. Its penetration of the soil in the vapour phase is now dependent on a number of important factors. Rising temperatures increase the vapour pressure and promote penetration. Due to the fact that penetration is only effected after some time, the period of influence also has an important bearing on the result of the treatment.

This period of influence is curtailed if for the purpose of minimising the waiting period, the ground is ventilated sometime after treatment i.e., if the surface is disturbed or the watershield eliminated. Furthermore, the soil sterilant vapours will be absorbed by the soil surface. Finally, a concentration slope will be formed, falling away from the point of application and a certain - however, constantly changing - balance between adsorption of the product and desorption, vapour pressure, and sterilant content of the soil air will be created. These circumstances are necessarily changing because the soil sterilant is steadily evaporating from the soil surface into the atmosphere thus steadily decreasing the amount of sterilant remaining in the soil. For these reasons, the type of ground and depth of top-soil (from the adsorption point of view) cannot be ignored. For example, the products penetrate much easier into structured silt than into compost soil as shown clearly in Table 1.

Fig. 1: Spontaneous penetration of Metham-Na into the soil in relation to soil temperature, reaction time, dosage (grs per sq. metre) and relative water saturation of the soil.

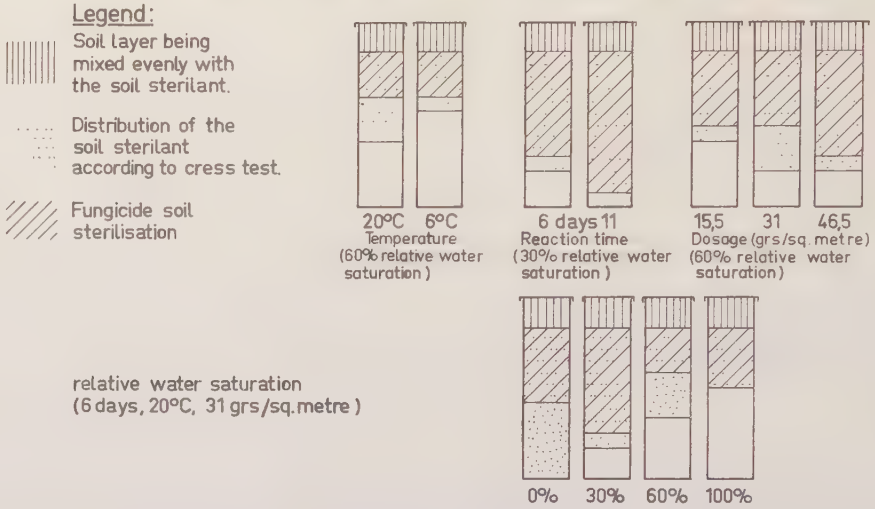

Legend:
Soil layer being mixed evenly with the soil sterilant.
Distribution of the soil sterilant according to cress test.
Fungicide soil sterilisation
20°C 6°C
Temperature (60% relative water saturation)
6 days 11
Reaction time (30% relative water saturation)
15,5 31 46,5
Dosage (grs/sq. metre) (60% relative water saturation)
relative water saturation (6 days, 20°C, 31 grs/sq. metre)
0% 30% 60% 100%

Table 1
Fungus mortality in percent after surface treatment with 31 g. Metham-Na (I) and 25 g. Methyl isothiocyanate (II) with subsequent spontaneous penetration of the substances into the soil. Relative water saturation 40%; test fungus Fusarium culmorum

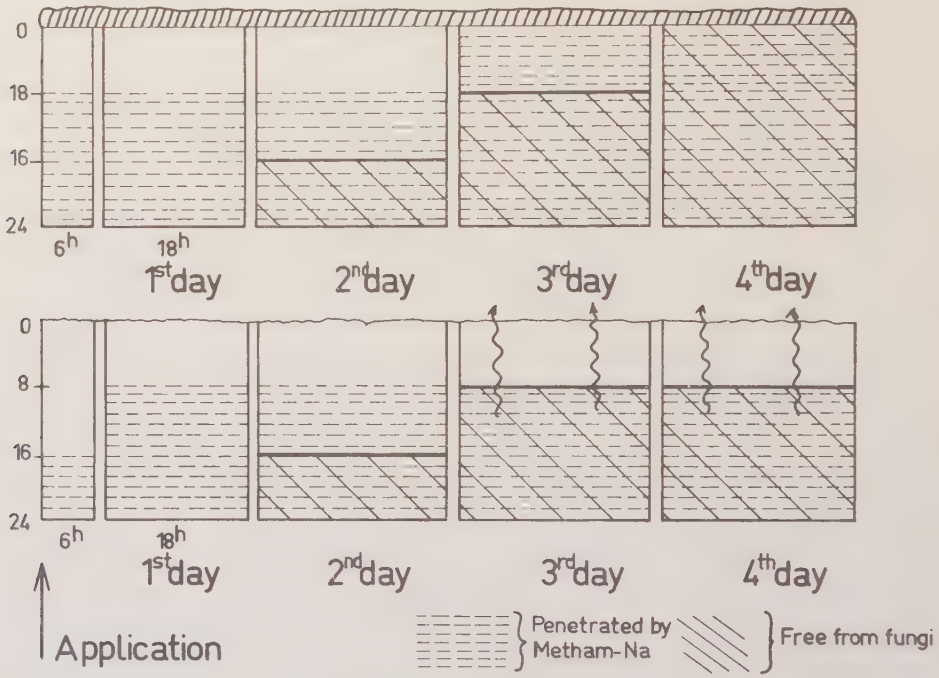
Temperature		6° C						20° C			
Soil type		compost			silt			compost		silt	
Substance		check	I	II	check	I	II	I	II	I	II
Reaction time 3 days											
soil depth in cm.	5	0	17	17	0	100	100	100	100	100	100
	10	0	0	0	0	100	0	83	100	100	100
	15	0	0	0	0	0	0	0	0	100	100
	20	0	0	0	0	0	0	0	0	100	100
	25	0	0	0	0	0	0	0	0	100	100
	30	0	0	0	0	0	0	0	0	100	100
Reaction time 9 days											
soil depth in cm.	5	0	100	100	0	100	100	100	100	100	100
	10	0	83	100	0	100	100	100	100	100	100
	15	0	50	0	0	100	100	100	100	100	100
	20	0	0	0	0	100	100	0	0	100	100
	25	0	0	0	0	100	100	0	0	100	100
	30	0	0	0	0	100	100	0	0	100	100

In this connection also the soil humidity plays an important part because a humid surface adsorbs gases much more readily than a dry one. Penetration is impeded by an increasing water saturation of the soil. Practically, the best penetration effect is obtained in dry soil. This does, however, not mean that the best fungicidal efficacy must also be obtained in completely dry soil because the soil fungi in this case occur in resistant forms (e.g. sclerotiae). It follows naturally that penetration of the soil can be improved by increased dosage (Fig. 1).

The herbicidal effect on cress seeds was in our experiments always found earlier than the fungicidal effect. According to our experience it is necessary that the product acts on the fungus substrate for at least 3 days at a toxic concentration. This condition is particularly important for the sterilisation of the uppermost soil layers when using the plough sole or injection methods. The evaporation of the product at lower levels takes place more slowly than its evaporation into the atmosphere (Fig. 2). On this basis it is possible that in the uppermost well ventilated soil layers the necessary fungitoxic concentrations can at no time be achieved and the sterilisation effect is insufficient. In order to prevent this, one can cover the soil with mats, tarpaulins or foils or by using a water seal during the period of activity. The water seal consists of a soil layer of about 1 inch thickness, being kept humid and in which the ascending sterilant vapours are adsorbed.

Fig: 2

The dependency of soil sterilisation with Metham-Na on soil cover and reaction time.

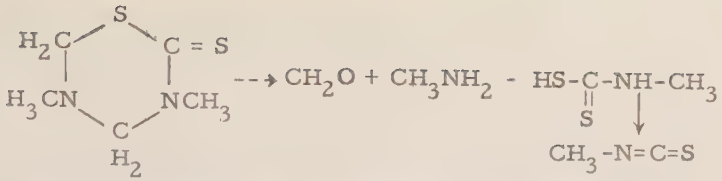


2. Chemical mechanism of action

With the theories of the action of the carbamates, isothiocyanate plays an important part as a fungicidally active break-down product. The effect of Metham-Na too, could easily be explained by a break-down to methyl-isothiocyanate as for example in the following form.

$$CH_3\text{-}\overset{H}{\underset{|}{N}}\text{-}\overset{S}{\overset{\|}{C}}\text{-S-Na} \quad - - \rightarrow \quad CH_3\text{-N=C=S} + NaSH$$

The same final product is also achieved with the break-down of 3,5-dimethyl-tetrahydro-1,3-5,2-H-thiadiazin-2-thione, however, with some intermediate steps as shown in the following formula:



As can be taken from the formulae the addition of water is necessary in the latter case. The break-down of Metham-Na however, based on the given formula would not require any water. Of course, catalytic factors such as soil organisms, mineral components and distribution over a large surface all participate in the break-down processes.

Due to the fact that all these considerations are hypotheses we were interested to find material for their support or refutation. The first foot-hold was gained by an exact comparison of the compounds Metham-Na and MITC. From the molecular weights of both substances, it follows that from one part Metham-Na not more than 0.57 parts MITC can be formed. In a soil sterilisation experiment with the test fungus Fusarium culmorum we tested the efficacy of MITC by using this material as such and in the form of the corresponding quantity of Metham-Na respectively. The graph of the test result shows that the quantity of MITC which possibly is formed by Metham-Na is 2.5 times more active than the 'straight' MITC. If the break-down hypothesis for Metham-Na were correct, the opposite result would be expected, assuming that the supposed active break-down product of Metham-Na had to be formed first. This finding is in conflict with the hypothesis we mentioned earlier. At least this cannot be the only principle of action. Moreover, Metham-Na must have a strong fungicidal efficacy of its own.

This result having been found in a soil sterilisation test, could be confirmed by a spore germination test with Alternaria tenuis. When carrying out the spore germination test, we encountered some difficulty because MITC is not water-soluble and had to be emulsified by addition of Tween 80 (Fig. 4). The ED-values (Effective Dosage) amount to 129 p.p.m. for MITC in direct use, but to 4 p.p.m. in the form of a corresponding quantity of Metham-Na. This astonishingly large difference in Ed-values has probably been caused by the water insolubility of MITC. However, it is still our opinion that this experiment proves that the sterilant action of Metham-Na does not depend only on its break-down to MITC.

These results explain the fact that under practical conditions about 40 per cent more MITC as such must be used, to achieve the biological efficacy of a given quantity of Metham-Na.

Because Metham-Na is marketed in the form of a water solution and usually has to be further diluted with water by the user (except in cases when accurate dispensing equipment is available), many held the view that the addition of water was definitely required to ensure the efficacy of Metham-Na. We have been carrying out additional soil sterilisation experiments to clarify this point. As test fungus Fusarium culmorum was

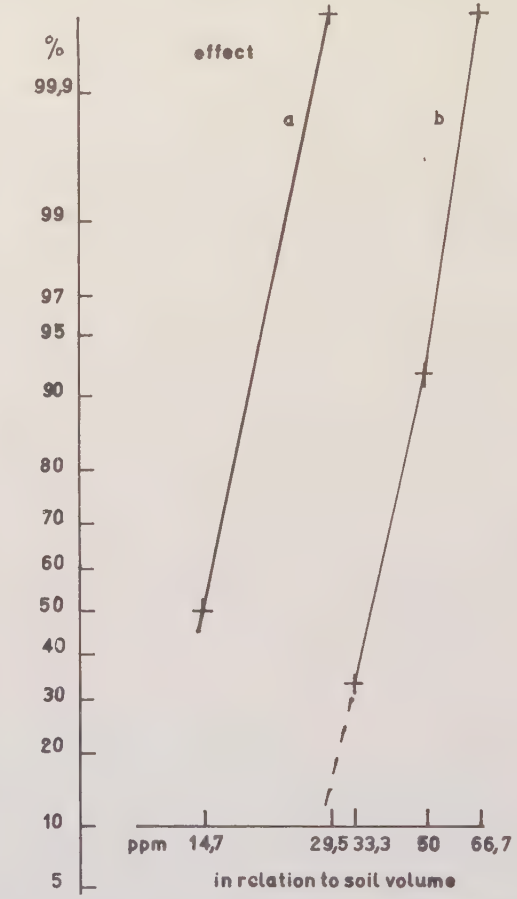


Fig. 3: Dosage-response curves for methyl-isothiocyanate in the soil sterilisation test.

a) in form of indirect use of Metham-Na

b) in form of direct application of methyl-isothiocyanate

again used. Silt was used in dry form and in saturated form to 30% of its relative water capacity, 100 ml. of 31% Metham-Na formulation and 125 ml. of a 20% MITC formulation were applied to the soil. The soil temperature during the experiment amounted to 6° C, the material acted 3 days. The Metham-Na formulation was not additionally diluted with water.

The result of this experiment is shown in Table 2.

Table 2
Fungus mortality and inhibition of seed germination in percent after sole treatment with 31 g. Metham-Na (I) and 25 g. Methyl isothiocyanate (II) with subsequent spontaneous penetration of the soil.

Test fungus: Fusarium culmorum; soil type: loam; soil temperature: 6° C; reaction time: 3 days; seeds: Lepidium sativum; applied undiluted.

		Fungus mortality						Inhibition of seed germination					
Water saturation		0%			30%			0%			30%		
Substance		check	I	II	check	I	II	check	I	II	check	I	II
Soil depth in cm.	5	0	100	0	0	0	0	0	100	100	0	76	28
	10	0	100	33	0	0	0	0	100	100	0	100	96
	15	0	100	100	0	100	17	0	100	100	0	100	100
	20	0	100	100	0	100	100	0	100	100	0	100	100
	25	0	100	100	0	100	100	0	100	100	0	100	100
	30	0	100	100	0	100	100	-	-	-	-	-	-

It can be seen that the action of Metham-Na is in no way hindered in air-dry soil. One can even detect a slight superiority of the formulation over the MITC formulation as far as the speed of soil penetration is concerned. As has already been shown a 30% relative water saturation causes a reduction of the soil penetration with both products. We can consequently state that both Metham-Na and MITC do not need any additional water for their effect. In this respect, these soil sterilants differ considerably from the third mentioned, viz. DMTT which is not broken down in the soil without water. A lack of water in the soil leads not only to an insufficient sterilisation, but also to a considerable prolongation of the waiting time.

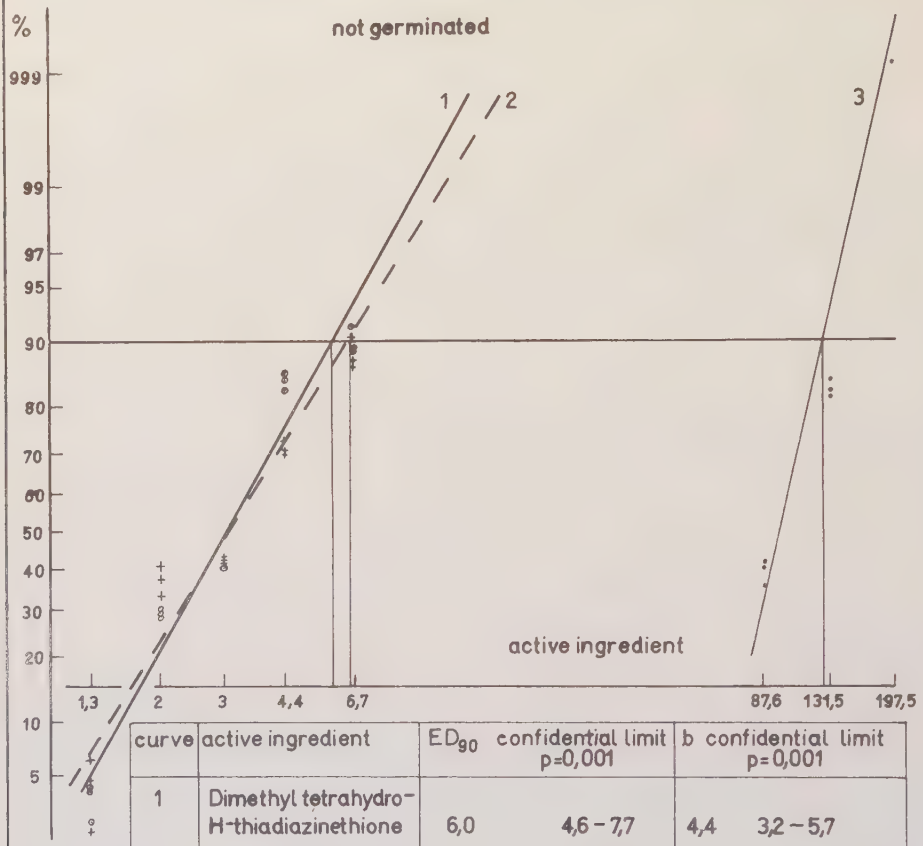
IV Practical Use

1. Methods of application

There are different methods of application available in the use of total soil sterilants.

a) 'Drenching method'

With the 'drenching method' the formulation is applied dissolved in water at about 5 litres wash per square metre of surface area. Thereafter the soil can be watered further. As our experiments have however



curve	active ingredient	ED_{90}	confidential limit p=0,001	b	confidential limit p=0,001
1	Dimethyl tetrahydro-H-thiadiazinethione	6,0	4,6 – 7,7	4,4	3,2 – 5,7
2	Na-N-methyldithio-carbamate	6,7	5,0 – 8,9	3,9	2,8 – 5,1
3	Methylisothio-cyanate	128,7	111,7 – 148,4	10,6	6,0 – 15,3

ED_{90} = effective dosis for 90 % inhibition of germination

Fig.4: Dosis response of the spore germination test

shown, the product is not transported deep into the soil as the water penetrates but is previously absorbed in the upper-most layers. From here it must penetrate on its own by means of its vapour pressure. Subsequent watering can consequently only be of use to provide a water-seal which prevents a quick release of the product into the atmosphere. Mechanical covering of the soil for an appropriate period increases efficacy. The penetration of the soil - as explained earlier - depends on several factors, and for this reason, the 'drenching method' is unreliable. It should only be used when applying a product with a high vapour pressure and which is water-soluble, as in the case of Metham-Na.

b) 'Plough sole' method

As the term indicates, with this method the product is applied either concentrated or diluted with water into the trough of the furrow or trench before the next furrow is turned, covering the treated soil. Here again, the substance must penetrate the soil unassisted. The efficacy of this method therefore depends additionally on the soil structure. On not too heavy, friable and crumbling soils, where the plough fills in the furrows evenly, the 'plough sole' method can be used with good success. On heavy humid soils where the soil does not crumble, but falls unbroken into the furrow, only partial success can be expected. A pre-requisite for this method is again a product with a high vapour pressure. (Metham-Na and MITC).

c) Injection method

The Injection method corresponds in some degree to the 'Plough sole' method as far as the provisions and the mode of action is concerned. Again the substance is brought into the deepest part of the soil to be treated and must penetrate the soil vertically and horizontally.

d) Intermixing method

When using the Intermixing method, the product is applied on the surface and immediately thereafter distributed throughout the layer of soil to be sterilised. Usually a rotatiller is used for this purpose. The rotatiller can be coupled to a Fertiliser scatterer or other dispensing apparatus so that the work can be done in one operation. With this method it is not essential that the substance itself has penetrative powers. It is therefore the surest method as far as effect is concerned, and furthermore products with a lower vapour pressure can also be used. It is however, recommended with this method that the ground be covered by a water-seal or with some kind of sheeting if possible. Suitable products: Metham-Na and DMTT.

2. Characteristics with regard to application of the products

Metham-Na can be used in concentration or diluted with water in all 4 methods of application. The formulations are liquid. An unpleasant irritating smell does under some circumstances make the application difficult.

Methylisothiocyanate is made available as a solution in xylene and must be used undiluted. It is only suitable for two of the described methods of application: 'Plough sole' method and Injection method. This product also has an unpleasant smell.

Dimethyl-tetrahydro-H-thiadiazin-thione: This product must be applied in a water suspension, though under experimental conditions it can also be used dry - however, only by means of the Intermixing method. There is no unpleasant smell. The effect and waiting time are dependent on the prevalence of soil water.

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INVESTIGATIONS ON NEMATICIDAL ACTIVITY AND CROP RESPONSES TO CHEMICAL SOIL STERILANTS

by J. E. Peachey
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Soil sterilization by steam or heat is rapidly being replaced by the use of compounds added to the soil which release a fumigant gas. These compounds have broad but varying bands of biocidal activity. They require special methods of application, are sensitive to soil conditions and their lack of specificity makes estimation of kill and crop response difficult to analyse.

Soil sterilant trials against a variety of plant-parasitic nematodes were started in 1959, to compare different compounds and to adapt assay methods. Examples of interim results obtained are given below. Treatments were generally applied at the accepted doses.

FIELD TRIALS

POTATO-ROOT EELWORM (Heterodera rostochiensis)

Trials were laid down on an infested potato field at Woburn Experimental Farm, Bedfordshire. The soil is a sandy loam with 16% clay. Root infestation was used both as an indication of eelworm kill (Goodey, 1957) and as an attempt to evaluate the damage suffered by the potato plant from the invasion of the nematode larvae into the root system.

In the 1960 preliminary trials, 16 different fumigants were used, and of these, only compounds releasing methyl isothiocyanate were promising in terms of decreased root infestation, yield increase and low post-harvest population.

This year the more promising treatments and in addition methyl bromide were tested in replicated plots. A clear relationship emerged from the degree of larval invasion and yield increase. Methyl bromide applied in autumn gave the best overall effect with 98% decrease of root infestation over controls, and a yield of ware potatoes of over 11 tons per acre, compared to only 0.2 tons per acre from control plots, having a root infestation of 6000 larvae per gram of root. Methyl isothiocyanate, sodium methyl dithiocarbamate and dimethyl tetrahydrothiadiazinethione gave decreases in infestation ranging from 91 - 98% and yields varying from 8 tons per acre following autumn treatment to 6 tons per acre following spring treatments. Dichloropropene and dimethyl thiuram disulphide gave 85% infestation decreases and yields of only 3 tons per acre. A substantial decrease in the numbers of eelworm larvae penetrating the root system was required to produce a reasonable yield. Past experience has shown that decreases below 90% are followed by rapid eelworm build-up.

Grainger (1954) has pointed out that effective yield increases can best be obtained by treatment of soils only moderately infested with potato-root eelworm.

The value of these results is in their relevance to tomato growing in infested glasshouse soils, rather than in the outdoor treatment of potato land where cost of treatment is far too high. These trials are therefore, to be repeated in 1961-62 in co-operation with the advisory service, on eight different glasshouse soils and are to involve eelworm assay by all known methods, measurements of soil ammonia-nitrogen after treatment and other disease control estimation.

VIRUS-CARRYING NEMATODE

Trials were laid down in Pembrokeshire on strawberry-growing land against Xiphinema diversicaudatum. The loam soil has 14% clay and is kept in good tilth. In 1960 adequate control of Xiphinema was obtained with the dichloropropene nematicide which gave 50% yield increases and a decrease in the incidence of Arabis mosaic virus. The methyl isothiocyanate liberators failed to give good Xiphinema control probably due to poor penetration.

In 1961, after treatment with the dichloropropene fumigant at 800 lb per acre or methyl bromide at 1 lb per 50 sq.ft, no living Xiphinemas were found. Dichloropropene at 400 lb per acre was 97% effective, but methyl isothiocyanate gave only 54% kill. The methyl bromide was shown to penetrate to 12 in. depth reaching a C. T. P. of 1000 mg./l./hr.

Increase in ammonia-nitrogen resulted from all treatments reaching 63 p.p.m. for methyl bromide, a ten-fold increase over control plots. The direct effect of these values on 1962 yields will have to be considered.

GENERAL OBSERVATIONS

The methyl isothiocyanate liberators have been shown to be highly active against potato-root eelworm but not so effective as dichloropropene against Xiphinema in the two soil types described. Methyl bromide was equally effective and is less sensitive to soil conditions, but its high mammalian toxicity and the need for gas tight seals makes it less attractive than the other nematicides.

The removal of trash following the previous infested crop, the production of a good tilth and careful application at correct temperatures and moistures, followed by rolling and watering, give the normal soil fumigants the best chance of working efficiently. This must be followed by adequate aeration of the soil before planting. The problems of fumigating tremendous volumes of soil by chemical sterilants remain formidable. To help overcome some of these problems these lines of work are indicated:- (a), the mechanisation of the whole application process; (b), further study of the theoretical and practical problems of different fumigants and their performance under different soil conditions; (c), improvement of assay techniques and the understanding of side effects; (d) the development of a new approach to nematode control, which avoids the necessity of treating the soil en masse.

I wish to acknowledge help given by Dr. B. D. Harrison, Mr. J. K. R. Gasser, Mr. G. N. Rao and the manufacturers of EMBAFUME C, TRAPEX, VAPAM, MYLONE, TRIDIPAM and D-D soil fumigants.

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SIX YEARS EXPERIENCE WITH METHAM-NA AS A SOIL FUMIGANT IN THE NETHERLANDS

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After its introduction in 1956, Metham-Na has found a large field of application in several horticultural crops. The following is a general view, seen from a practical standpoint of experience with this fumigant in Dutch horticulture. This experience was obtained from many field tests carried out by several Research Institutes and by our own organisation as well as from results of commercial use.

Brief consideration is given to the effectiveness of the chemical against the principal plant parasitic organisms and to techniques of application. Finally, a summary is given of the recommendations for practical use on various crops.

ACTION AGAINST NEMATODES

Although Metham-Na is effective against several nematode-species, its greatest practical use is for the control of free living species. Dosage rates from 50 ml. per square metre on very light sandy soils to 75-100 ml. for heavier soils give excellent results for at least two seasons. For this purpose the fumigant finds practical application for lilies, strawberries and various outdoor grown vegetables. The action against cyst-forming nematodes lacks residual effect, and may not suffice for one season (carrots).

Although Metham-Na undoubtedly gives a positive effect against the root knot nematode, Meloidogyne marioni, results of practical application vary. As yet there is no uniform opinion about the reasons for those cases where the chemical failed. Up till now for tomatoes in glasshouses Metham-Na has only been recommended when the infestation with root knot eelworm in the foregoing season was light to moderate. In cases of serious infestation more specific nematocides such as DD, Telone or EDB are preferred, Metham-Na is not recommended for use on cucumbers.

ACTION AGAINST SOIL FUNGI

The action of Metham-Na against soil fungi is the most interesting aspect of its use. It has found a wide field of application in the bulb industry against soil borne Fusarium-diseases (hyacinths, iris). Furthermore it has proved to be a usable fumigant for the prevention of damage by the vascular disease Phialophora cinerescens in carnations and "corky root" in tomatoes (Mycelia sterilia). Dosage rates for an economic use against fungi lay in the range of 75-100 ml. per square metre.

In some commercial applications as well as in a detailed field test it was concluded that rates of 75 and 100 ml. per square metre gave a considerable reduction of Verticillium Wilt in tomatoes. However the impression remains that higher rates are desirable for optimal control of

this disease. In a replicated test against this disease in Chrysanthemums, chloropicrin was far superior. Finally, the control of damping off diseases in seed beds (flowercrops, conifers, brassicas, tobacco) coupled with a more vigorous development of young plants has been recognized as a welcome cultural practice.

ACTION AGAINST WEEDS

A long residual action against many annual and some perennial weeds is seen by many growers as an extra advantage of an application with Metham-Na. A good response in weed control, however, depends strongly on the application technique. Too much watering immediately after application often reduces the effect, whereas sometimes failures are shown after mechanical injection. After application in outdoor soils high rainfall may be the cause why weedseeds in the upper soil layers escape from the chemical. Clods and undecayed roots will also prevent an even distribution of the chemical through the soil. The effectiveness of Metham-Na against quack grass, Agropyron repens is worth mentioning. Although for most crops its exclusive use as a herbicide is too costly, Metham-Na has recently found a wide use as a herbicide in seedbeds, especially in those crops where handweeding is impossible or too costly (flowers, cauliflower, lettuce). In these cases a dosage rate of 20 ml. per square metre applied 5 - 7 days before seeding or planting as a drench with reduced amounts of water gives a sufficiently long residual effect against annual weeds.

APPLICATION TECHNIQUES

After much experimental work had been carried out it was concluded that, for most crops, the drenching technique is to be preferred to mechanical injection. The required dosage rate is diluted with a 10 - 15 fold amount of water and evenly sprinkled on the surface of the soil. Immediately after this application the chemical has to be watered in with a relatively large amount of water. In working out a recommendation for this technique it has been found very difficult to indicate precisely an amount of water necessary for a sufficiently deep penetration of the chemical into the soil, because the rate of penetration differs so much in different soil and moisture conditions.

As each grower knows the water capacity of his soil best, we believe that only the grower himself is capable of judging the amounts of water which are needed to reach a penetration of about 12 inches deep.

In some cases the treatment was a complete failure because of the escape of the diluted chemical through the drains. Other cases of failure were caused by a too high water table during application. Attempts to replace watering-in by plastic covers after sprinkling the chemical on the soil surface have not yet led to alteration of the present-day recommendations. Watering-in is with a hose or with an overhead sprinkler irrigation system. Recently some progressive growers started applying Metham-Na by way of their sprinkler irrigation system, but care is necessary for some kinds of paint may be affected by the chemical.

To the statement that the drenching technique is to be preferred for most crops, two exceptions must be mentioned. The first is treatment against a root rot disease in hyacinths, where mechanical injection proved superior to drenching. The second exception applies to the treatment of tomato greenhouses where, for the past two years, both techniques have been practised. Although results seem to indicate a slight superiority of the drenching technique, more experience is needed before a choice of recommendations is made.

PHYTOTOXICITY

Relatively few cases of serious phytotoxicity after commercial application have been reported. With a waiting period of 3 - 4 weeks between application and planting, the method proved to be safe for bulbcrops during August till the beginning of October. For vegetable crops average waiting periods are necessary of 2 - 3 weeks after applications in August, 3 - 4 weeks after applications in September and 4 - 6 weeks after applications in October. Treatments with Metham-Na are only recommended when soil temperature is above 50° F. The above mentioned data mainly refer to the lighter soil types which are poor in organic matter. In soils rich in organic matter a safe waiting period may sometimes be extended to 8 weeks.

Although the fumes of Metham-Na are less harmful than those of chloropicrin, special attention must be given to prevent damage to neighbouring living plants.

PRESENT DAY USE and RECOMMENDATIONS

ORNAMENTAL CROPS

Carnations

After successful trials by the Experiment Station at Aalsmeer, Metham-Na has found a wide use in this crop for control of the vascular disease Phialophora cinerescens, see Table 1. The drenching technique at a rate of 100 ml. per square metre is preferred over mechanical injection. On heavy soils a split treatment is carried out, beginning with a treatment of the subsoil with the upper soil removed; afterwards the upper layer of soil is treated separately. Further tests are needed before a recommendation can be given re Fusarium oxysporum.

Hyacinths

Metham-Na has been widely used during the past 4 years against a root rot disease caused by Fusarium culmorum. At rates of 75 - 100 ml. per square metre only mechanical injection is recommended 3 - 4 weekss before planting. This treatment must be supplemented by a drench of formaldehyde in the seedbed just prior to planting at a depth of 8 inches . (See Table 2). Heavy rains, shortly after treatment, may reduce efficiency.

Bulbous Iris

Metham-Na has proved effective against a root rot disease, probably

caused by a Fusarium sp. Unlike the case of hyacinths, mechamical injection gave inferior results compared to drench applications. (See Table 3). An additional treatment with formaldehyde did not improve the effectiveness. The same difficulties after heavy autumn-rains may be expected as mentioned under hyacinths.

Hippeastrum (Amaryllis) in glasshouses

Against a serious root rot disease in this crop, Metham-Na gave satisfactory results on a number of vineries. Applied on the drenching technique at rates of 100 - 125 ml. per square metre the fumigant gave results comparable to those of steam sterilisation.

Lilies

Metham-Na is used by several growers to prevent a root rot in which free living nematodes play an important part. The drenching technique is used at rates of 50 - 75 ml. per square metre. (See Table 4).

Seedbeds of flowercrops and nurserycrops

Treatment of seedbeds is becoming very popular. Drench applications with relatively small amounts of water are made 7 - 10 days before seeding: (a) for weed control purposes in those crops where mechanical weeding may cause serious damage to the roots. A dosage rate of 20 ml. per square metre gives a sufficiently long residual effect. (b) to prevent damping-off diseases with additional weed control. For this application a rate of 75 ml. per square metre is necessary.

Treatment of potting soil

Metham-Na may be cheaper to use than chloropicrin, and trial applications to layers of soil, 8 inches high, at a rate of 100 ml. per square metre are recommended.

VEGETABLES and FRUIT

Strawberries

Metham-Na proved very effective against black root rot. On very light sandy soils it is applied at a rate of 50 ml. per square metre; on heavier soils or on soils with a higher content of organic matter an increased rate of 75 up to 100 ml. per square metre is desirable. No data are available about the effectiveness against Verticillium Wilt.

Carrots

Striking improvements in growth may be obtained after treatment with Metham-Na, but several cases of failure have been repeated. It is concluded that a recommendation for the use of Metham-Na on carrots can only be given when certain species of free living nematodes are the main

cause of growth reduction, in which case a response to the treatment has been observed for two subsequent years.

Lettuce, endive, radish, beans

Although an exclusive soil fumigation for these crops will seldom be carried out, mention must be made of the favourable responses after a Metham-treatment, especially on light soils (Table 5).

Fumigation often leads to a remarkable acceleration in yield (Table 6), which may be of great importance in catch crops.

Seedbeds

The attractiveness of treating seedbeds with Metham-Na has already been pointed out. A treatment of seedbeds for brassica crops has become an established practice at a rate of 75 ml. per square metre. This year some growers have used 20 ml. per square metre 6 days before planting out lettuce (in soil blocks) for the purpose of weed control.

Tomatoes (and lettuce) in glasshouses

Dutch growers take a great interest in the application of Metham-Na for the following reasons: (a) Metham-Na offers less danger for neighbouring crops than chloropicrin; (b) as contrasted with chloropicrin, Metham-Na has a favourable effect on lettuce when grown as a catch crop for tomatoes; (c) Metham-Na can be applied by the growers themselves.

Table 1

Soil fumigation against Phialophora cinerescens in carnations

Data from G. Scholten, Exp. Station at Aalsmeer.
Treatments made in April, 1956. Planted 3 weeks later.

Number of diseased plants	Oct. 15	Nov. 18	Dec. 31	Jan. 27	Feb. 27
formaldehyde	1	2	4	6	14
mercury compound	8	10	20	32	51
control	14	17	22	33	43
copper-fungicide	4	15	28	41	51
Metham-Na	0	0	0	0	0

Table 2

Soil fumigation against Fusarium culmorum in hyacinths

1. Trial in 4 replicates at van Zanten Bros - Hillegom.
Application, September 17 - 1957. Planted October 18. Var. Pink Pearl.
Plotsize $\frac{1}{2}$ bed (+ 7 square metres).

Treatment	Yield increase in % per 4 replicates
Control	163
Metham-Na 75 ml/m^2 injection	194
Metham-Na 100 ml/m^2 injection	203
Metham-Na 75 ml/m^2 injection + formaldehyde drench	290

2. Trial in 3 replicates at van Zanten Bros - Hillegom.
Early treatments with Metham-Na on September 16, 1960
Late treatments with Metham-Na on October 17, 1960
Date of planting and treatment with formaldehyde November 23, 1960.

Treatment	Yield increase in % per 3 replicates
Control	99,0
Metham-Na 100 ml/m^2 early injection + formaldehyde	132,6
Metham-Na 100 ml/m^2 drench method, early application	98,9
Metham-Na 100 ml/m^2 late injection	91,5
Metham-Na 100 ml/m^2 late injection + formaldehyde	129,1
Metham-Na 100 ml/m^2 drench method, late application	85,6

Table 3

Soil fumigation against root rot in bulbous Iris

Summary from a trial carried out in cooperation with the Advisory Service at Amsterdam. Var. White Excelsior.

Treatments	Yield in kg.
Control	12,70
Metham-Na injection, 50 ml/m^2	12,75
Metham-Na injection, 75 ml/m^2	14,05
Metham-Na drench, 50 ml/m^2 (+ 10 l. of water)	16,15
Metham-Na drench, 75 ml/m^2 (+ 10 l. of water)	17,90

Table 4

Soil fumigation against free living nematodes in Lilium speciosum rubrum.

Summary from trial carried out in cooperation with the Advisory Service at Heemsker. Treatment on December 10, 1958. Date of planting March 5, 1959.

Treatments	Yield in kg			
	repl. a	repl. b	repl. c	total
Metham-Na, 50 ml + 2 l. of water/m^2	11,8	11,2	11,7	34,7
Metham-Na, 50 ml + 5 l. of water/m^2	12,8	12,0	12,0	36,8
Control	10,6	10,1	10,3	31,0
Metham-Na, 50 ml/m^2 injection	13,1	11,6	12,7	37,4
Metham-Na, 50 ml/m^2 drench + 10 l. of water	12,5	13,5	12,8	38,8

Table 5

Soil fumigation in strawberries, lettuce and carrots

Influence of different amounts of water given after a treatment with Metham-Na. Published in Annual Report 1959 of the Exp. Station at Heemskerk.

	lettuce number of heads	carrots number of branches	strawberries yield in kg/ 100 sq. metres
Control	100	100	159
Intercropping of Tagetes	-	-	171
Metham-Na, 50 ml. + 5 l. of water/m^2	193	153	207
Metham-Na, 50 ml. + 20 l. of water/m^2	203	200	228
Metham-Na, 50 ml. + 40 l. of water/m^2	219	194	234
Metham-Na, 75 ml. + 20 l. of water/m^2	229	197	220
Metham-Na, 75 ml. + 40 l. of water/m^2	235	197	224

Table 6

Acceleration in yield after a treatment with Metham-Na for radish

From a trial carried out by the Advisory Service at Gronigen.
Treatment on November 14, 1959. Radish sown on November 28, 1959.

date	Number of bunches per 100 sq. metres	
	from treated plots	from untreated plots
March, 23	60	28
March, 28	140	101
March, 31	70	82
April, 4	127	140

SOME RECENT INVESTIGATIONS WITH CHEMICAL SOIL STERILANTS

by W. H. Read, J. T. Hughes and R. J. Smith
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Introduction

The high cost of sterilising glasshouse soils by steaming has increased the interest in alternative chemical methods. Formaldehyde and certain other chemicals have been used for many years for the treatment of soils which have become less fertile due to the successive growth of like crops. These chemicals have been found, in practice, to give results inferior to those obtained by steaming, even in the absence of soil-borne plant-parasitic nematodes, and often continued use leads to a diminishing response.

Experiments have been made to find if restoration of the fertility of the soil in glasshouses, which has fallen due to cropping with tomatoes for a number of years, can be maintained by annual soil treatments with chloropicrin or metham-sodium (sodium N-methyl-dithiocarbamate).

Tomato plants may become infected with tobacco mosaic virus (TMV) which has persisted in the soil over winter in debris from a previous infected crop. In view of the reduction in crop and fruit quality caused by this virus, knowledge of the effect of the sterilants on the incidence of soil-borne virus is needed.

Sterilisation of many soils by steam causes a considerable increase in the availability of manganese, which may result in manganese toxicity in the tomatoes. Although manganese toxicity can usually be prevented by liming the soil, its possible causation by steaming is a slight disadvantage. Information on the availability of manganese after chloropicrin and metham-sodium treatments has to be sought.

The results of trials commenced in 1958 showed the value of metham-sodium in restoring the fertility of a 'sick' tomato soil, but in view of the incidence of damage on commercial nurseries, experiments were also commenced to obtain information on the distribution of the active chemical methyl isothiocyanate, resulting from the decomposition of metham-sodium in the soil, and the nature of and reason for the occasional, unpredictable injury to crops planted after the application of metham-Na. The role of methyl isothiocyanate in producing certain abnormalities in the growth of tomatoes has been recorded by Hunnam and Waddington (1961).

I. Glasshouse Cropping Trials

Experimental

General design

In these trials the layout was a latin square of the four treatments, steaming, chloropicrin, metham-Na and unsterilised, and was accom-

modated in two contiguous non-partitioned glasshouses.

These glasshouses had been cropped with tomatoes for five successive years without sterilisation of the soil. Hence the condition of the plants in 1958 indicated that sterilisation was required. Examination of the roots at the end of the 1958 crop showed they were extensively rotted and 'corky'. Very little Colletotrichum atramentarium, often present in old tomato soils, was present and there was no acute disease such as Verticillium wilt, or infection by root-knot or potato root eelworm in 1958.

The plots were 25' x 5' and 48 plants were grown in each plot. Plastic sheet barriers were inserted between the plots to a depth of 2 ft. The soil was a fine sandy loam which readily became compacted when watered.

Sterilisation procedures

(1) Steaming

The soil was steamed in the normal manner to maintain a temperature of 110° F throughout the soil to a depth of 12" for 20 mins.

(2) Chloropicrin

3.5 ml. amounts were injected at 12" staggered spacings at a depth of 6", (equivalent to 540 lb/acre), followed immediately by 'sealing' the surface by treading and watering with about $\frac{1}{2}$ gal./sq.yd. This light watering was repeated on two occasions during the ten days following the dates of application: 12/11/1958, 5/11/1959 and 1/11/1960.

(3) Metham-Na

A 1 in 200 dilution of a proprietary concentrate, found on analysis to contain 39% of sodium N-methyldithiocarbamate, was uniformly applied as a drench at the rate of $4\frac{1}{2}$ gals./sq.yd.

Dates of application: 13/12/1958, 14/10/1959, 31/10/1960.

All the plots were thoroughly cultivated before sterilisation, and the chemically treated plots were forked over twice before flooding with water prior to planting.

Planting dates were 6/3/1959, 4/3/1960 and 2/3/1961.

To meet the requirements of other research projects in 1959 each plot was planted with eight plants of six varieties of tomatoes. The varietal yields were recorded for each plot but the figures given in Table 1 are based on the total yields of the plots. Two varieties, Potentate, a non-vigorous variety which is readily affected by adverse soil conditions, and Moneymaker, a vigorous variety more tolerant of soil sickness, were grown in halves of each plot in 1960 and 1961.

Yields and grades of fruit were recorded and the effects of the treatments on root size, and the extent of corky and rotted root were assessed by visual examination. Assessment of the effect of the soil treatments on

root conditions, based on relatively simple physical measurements were found unsatisfactory.

Associated Investigations

(a) Soil-borne virus

The Plant Pathology section of the Institute collaborated in the 1960-61 trial and recorded the number of tomato plants which became infected with tobacco mosaic virus (TMV) via the soil. All plants were infected with TMV in September 1960. Stringent precautions were taken to prevent the plants becoming infected with TMV from sources other than the soil, within 4 weeks from planting. A full account of this work will be published elsewhere.

(b) Availability of manganese

The amounts of available manganese in the soil, and the manganese contents of plant tissue from plants grown in the variously treated soils were determined in 1961 by the Chemistry department of the Institute.

Results

Table 1

Yields of tomatoes - lb/plant

M - var. Moneymaker P - variety Potentate

	1959	1960		1961	
Treatment		M	P	M	P
Unsterilised	7.25	6.57	6.02	4.97	4.57
Steam	9.75	11.79	11.18	9.01	8.25
Chloropicrin	9.63	11.66	10.93	9.04	8.30
Metham-Na	9.48	11.25*	10.27*	8.35	7.81

* Treatments differing significantly from steam at P = 0.05. All the sterilisation treatments in 1959 and 1960 differ significantly from unsterilised at P = 0.01.

It has not been possible to analyse in detail the figures for the 1961 crop since harvesting was not completed until the end of September.

Table 2

Effects of treatments on TMV infection and availability of manganese

	Plants infected with TMV 4 weeks after planting	Manganese content of leaves p.p.m. dry wt.	Water soluble and exchangeable manganese in soil - p.p.m. in dry soil
Unsterilised	2	130	3.3
Steam	0	1160	43.0
Chloropicrin	19	400	8.9
Metham-Na	8	105	2.6

The results (Table 1) show that under the conditions of these trials there was no falling off in response to sterilisation with chloropicrin in comparison with the response to steam. In 1960 the effect on fruit yield of metham-sodium was significantly less than that of steam. The individual plot yields in 1961 suggest that both steam and chloropicrin gave significantly better results than metham-Na.

The response to all sterilisation treatments was most marked in the first half of the season. No treatment resulted in the roots remaining free from corkiness and rotting until their removal at the end of September. There were very wide variations in the size and condition of roots in all the plots, but when classified according to their freedom from corkiness and browned and rotted root, and the amount of clean fibrous root, the roots from the steamed plots were significantly better than those from other treatments.

The marked difference, early in the season, in the growth of plants in all the sterilised plots and those in the unsterilised plots, and the poor condition of the roots at the end of the season indicates re-infection of treated soils from the subsoil.

Studies of the distribution of methyl isothiocyanate in the plots treated with metham-sodium in 1959 and 1960 show that its concentration did not exceed 3 p.p.m. at a depth of 15 - 20 ins. and 10 p.p.m. at 10 - 15 ins. It is apparent from the numbers of plants infected with soil-borne virus (Table 2) that steaming destroys or reduces the amount of virus. The greater incidence of TMV in the chloropicrin and metham-sodium treated plots is probably attributable to the effect of these sterilants on the soil organisms which rot plant residues, and the reduction in the rate of decomposition of plant residues delays the exposure of the virus to biological or other destructive action.

The availability of manganese in the soil (Table 2) is considerably increased by steaming but practically unaffected by Metham-Na. A slight increase resulted from treatment with chloropicrin.

II. Metham-Na - Phytotoxicity Studies with Tomatoes

In view of the many instances of damage following the application of metham-sodium in 1959 the phytotoxicity of this compound, and of the products which are known to be, or might be, produced by its decomposition, were studied. The decomposition of dilute solutions of metham-sodium in contact with soil was found to be so rapid (Hughes, 1960) that the investigations were restricted to an examination of the phytotoxicity of methyl isothiocyanate, N-methylthiourea (which might result from the interaction between the isothiocyanate and ammonia produced in the treated soil) and s -dimethylthiourea (a possible product of the interaction of methyl isothiocyanate and methylamine, which may conceivably be formed by the decomposition of methan-Na).

Tomato plants were grown in pots in sterilised soil to which N-methyl thiourea and s-dimethylthiourea were added. Concentrations of these compounds caused no visible toxicity when present in the soil at 25 p.p.m., and although some temporary marginal leaf scorch and chlorosis on young plants resulted at about 50-100 p.p.m., there was no permanent injury or plant growth modification resembling that which occasionally occurred in commercial houses treated with metham-sodium.

Methyl isothiocyanate, in similar tests, but with complete enclosure of the treated soils and pots to exclude vapour from the plants, caused severe permanent injury to tomato plants at concentrations exceeding 5 p.p.m. The damage from such soil applications was direct by killing the roots or causing the base of the stem to collapse, and growth modifications did not result at concentrations which did not cause direct damage.

There was evidence from cases of damage on commercial nurseries that damage resulted from a volatile substance, and tests were made in which plants were exposed to different concentrations of methyl isothiocyanate vapour in airtight chambers for varying periods.

Concentrations exceeding 0.3 g/m^3 caused severe scorching, wilting and sometimes death of the plants. Exposure for a single period of 17 hrs. to concentrations of 0.2 - 0.1 g/m^3 caused scorching of the lower leaves of tomatoes at the planting-out stage but no subsequent modifications of growth or other abnormalities. On the other hand similar plants exposed to concentrations of 0.05 - 0.01 g/m^3 for 17 hr. periods on 4 or more consecutive days subsequently developed abnormalities ranging from complete blindness due to the termination of the main stem at an abnormally large flower truss and the development of flower trusses instead of side shoots, to the formation of abnormally large flower trusses with long truss stems.

The first one or two trusses which became visible after prolonged exposure to methyl isothiocyanate vapour usually failed to develop, often producing only a few small, seedless fruits. Many of the abnormal flower trusses which presumably were in process of initiation at the time of exposure to methyl isothiocyanate, bore fertile flowers and produced normal fruit.

Tomato seedlings exposed to the low concentrations were distorted, stunted and frequently became blind.

There did not appear to be any differences in the susceptibilities of a large number of tomato varieties.

Conclusion

These results confirm the conclusion of Hunnam and Waddington (1961) that methyl isothiocyanate vapour is responsible for the phytotoxic effects which have occasionally followed the use of metham-Na in tomato houses.

In a glasshouse of average dimensions the release of 1 p.p.m. of methyl isothiocyanate from the soil to a depth of 1 ft. will provide 0.24 g/m^3 in the air assuming there are no air changes.

Studies of the rate of loss of methyl isothiocyanate from the soil in the glasshouses at this Institute have shown that a residual amount of several p.p.m. may be retained in the soil for at least six weeks. Organic and clay soils sorb methyl isothiocyanate more strongly than the sandy loam and residual amounts are almost certainly greater in practice in these soils.

It is necessary to obtain more information on the factors which influence the rate of loss of these strongly absorbed residues in order to devise means whereby the inconveniently long period between the application of methyl isothiocyanate, or substances producing this compound can be reduced.

III. The distribution of methyl isothiocyanate in metham-Na treated soil.

The amount of decayed and swollen roots on plants removed, at the end of the 1959 crop, from the metham-sodium treated plots in the comparative trial of this sterilant with chloropicrin and steam, described above, suggested that re-infection may have resulted from the failure of the methyl isothiocyanate to penetrate to a sufficient depth. The vertical distribution of methyl isothiocyanate following applications of metham-sodium to these plots in the 1960 and 1961 cropping trials was therefore studied.

In some of the trials made in tomato houses by Advisory Officers and others, the injection of metham-sodium concentrates has given better results than its application as a drench; in other trials drenches have proved superior. This may be due to some extent to differences in the pathogenic condition responsible for crop reduction or to differences in soil type and temperature, resulting in differences in the distribution of methyl isothiocyanate. Some experiments have been made at the G.C.R.I. on the distribution of methyl isothiocyanate resulting from alternative methods of applying metham-sodium, but further work is necessary to obtain a comprehensive picture of distribution in other glasshouse soils with alternative application methods, and of the accompanying problem of eliminating hazardous residues from such soils.

A number of workers have investigated the influence of various soil factors and application methods on the biological activity and efficiency of pest and disease control of metham-sodium and certain other compounds which also produce methyl isothiocyanate on decomposition. A comprehensive review of their findings is given by Lambe, who studied the influence of soil temperature and moisture, and the method of application of metham-sodium and 3,5-dimethyl-tetrahydro-1,3,5,2H - thiadiazine-2-thione on fungitoxicity.

Experimental

In all the experiments a commercial concentrate containing 39% of metham-Na was used.

(A) Glasshouse Cropping Trial, 1959-60.

1 pint of concentrate in 25 gallons of water per 50 sq. ft. applied as a drench.

Date of application	14.10.1959
Nature of soil	fine sandy loam
Moisture content 1959 = 18.4%	
Soil temperature at 6 ins. over a 10 day period following application	1959 58° F - 54° F

(B) Experiments with different methods of application

Each treatment was applied to 4 plots

(1) 1 pint of concentrate in $12\frac{1}{2}$ gals. of water per 50 sq. ft., followed immediately by watering with $12\frac{1}{2}$ gals. water per 50 sq. ft.

(2) 1 pint of concentrate in 2 gals. of water per 50 sq. ft., uniformly applied to the soil surface and immediately followed by watering with $12\frac{1}{2}$ gals. water per 50 sq. ft.

(3) 1 pint of concentrate per 50 sq. ft., injected at 11.30 ml. at a depth of 8 ins. at 12 in. rectangular spacings. Injection holes were sealed immediately after each injection with soil and the soil surface was sealed by watering with 5 gals. water per 50 sq. ft.

Samples of soil at 0-5", 5"-10" and 10"-15" depths were removed at random from all plots excepting those in which the metham-sodium was injected, where the samples were taken at points equidistant from four injection points and the sampling holes were firmly filled with soil.

The amounts of methyl isothiocyanate in these samples were determined by a method based on the distillation of the compound into a solution of ammonia and measurement of the absorption of the resulting methylthiourea at 235 m.μ(4).

Results

The amounts of methyl isothiocyanate at three different depths is shown graphically in diagrams 1 - 4.

It is apparent that in the soil treated in these experiments, the concentration of methyl isothiocyanate at a depth of 5-15 ins. is higher where the metham-sodium is applied in a relatively large volume of water (Figs. 1 and 2) than where a relatively large amount of water is applied immediately after the application of more concentrated metham-sodium solutions (Fig.3).

It is necessary to stress that current recommendations are that when metham-sodium is applied as the slightly diluted concentrate or by injection it should be thoroughly incorporated into the soil by rotary cultivation. Even so it is possible that such cultivation which will not exceed and may sometimes be less than 8 ins. may not ensure an adequate concentration of methyl isothiocyanate at depths below 8 ins.

The results obtained from the injected plots (Fig. 4) were unexpected. One possible explanation is that the decomposition of the metham-sodium was very slow due to its localised absorption in a small quantity of soil, and loss of methyl isothiocyanate by upwards diffusion or decomposition was sufficiently rapid to prevent the attainment of the expected concentration in the soil most remote from the points of injection. Such an explanation is consistent with the observation that the soil at or very close to the points of injection contained appreciable amounts of methyl isothiocyanate at least 10 weeks after application.

Incorporation of the metham-sodium with the soil by mechanical cultivation should ensure more uniform lateral distribution in the top eight inches but tests are necessary to find whether it appreciably affects the concentration in the soil below 8 - 10 ins.

Conclusions

The paper presented must be regarded as a progress report since much more experimentation is necessary before definite conclusions are possible.

The results of the three years' trial in tomato houses at the G. C. R. I. suggest that in a typical soil which has become less fertile by repeated cropping with tomatoes, annual applications of chloropicrin may be somewhat more effective than metham-sodium and equal to steam in maintaining fertility. Further experiments on similar lines are necessary to see whether this is the case with other soils and where other fungi such as Verticillium spp. Colletotrichum atramentarium are present.

It is known that chloropicrin does not give satisfactory control of root-knot and potato-root eelworms.

The disadvantage of both chloropicrin and metham-sodium is the long period which must elapse between application and planting. The phytotoxic effects of metham-Na on tomatoes result from traces of methyl isothio-

cyanate vapour in the air, and more knowledge of the factors affecting the rate of release of this compound, and its de-activation in soils, might enable this interval to be reduced.

Distribution studies show that with methods of application tried so far, the concentration of methyl isothiocyanate in the soil at depths of 12" or more does not exceed 5 p.p.m., and the condition of the roots at the end of the season suggests that recontamination of the top soil by organisms from the subsoil may be important with each of the three methods of application. The use of larger volumes of more dilute metham-Na than at present advised may increase the depth of effective sterilisation.

Injection of a 40% solution of metham-sodium into a moderately moist soil resulted in unsatisfactory distribution of methyl isothiocyanate. It is almost certain that rotary cultivation immediately after injection, as now advised, will greatly improve lateral distribution but at present it is impossible to say whether penetration beyond a depth of about 8 ins. will be improved.

Acknowledgement

We wish to thank Dr. L. Broadbent for permission to include the results of his work on the effect of the soil sterilisation treatments on the incidence of TMV infection arising from soil-borne virus, and Mr. J.H.L. Messing, also of this Institute, for permission to record the results he obtained from determinations of the manganese contents of leaves, and soluble and exchangeable manganese in the soils of the plots in the glasshouse trial. We also wish to thank Miss B.M. Finch for much valuable assistance.

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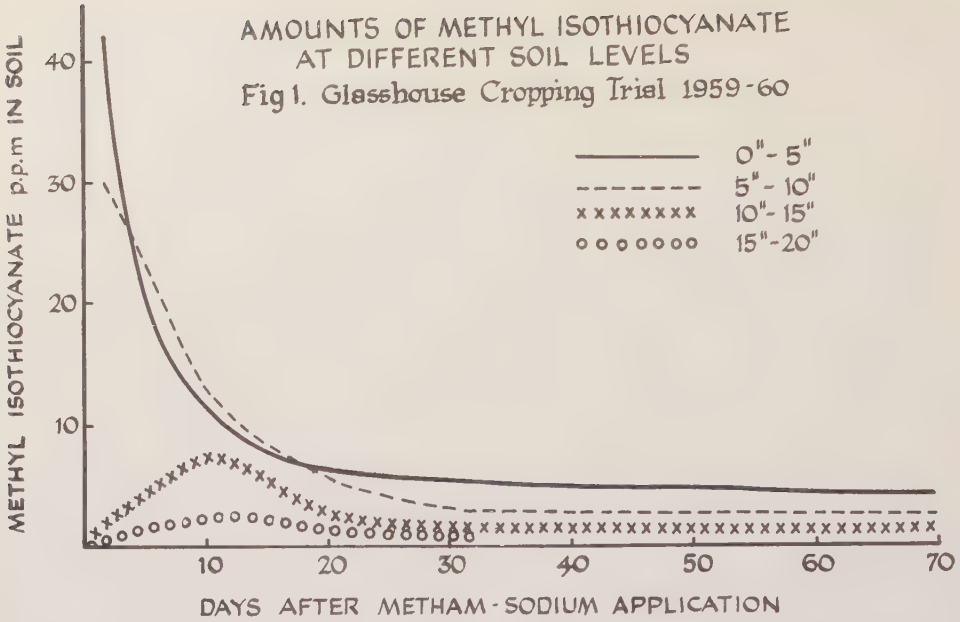
AMOUNTS OF METHYL ISOTHIOCYANATE
AT DIFFERENT SOIL LEVELS
Fig 1. Glasshouse Cropping Trial 1959-60
0"- 5"
5"- 10"
10"- 15"
15"-20"
METHYL ISOTHIOCYANATE p.p.m IN SOIL
40
30
20
10
10
20
30
40
50
60
70
DAYS AFTER METHAM-SODIUM APPLICATION

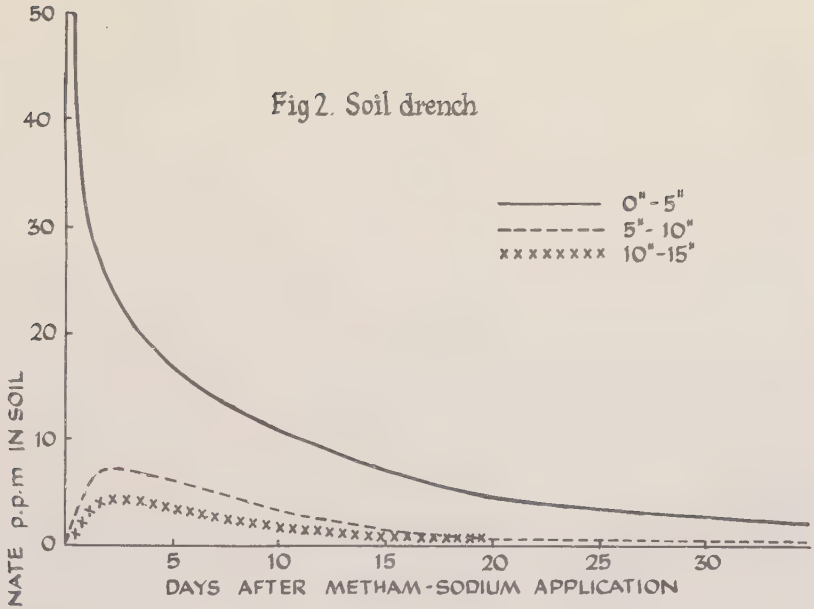

Fig 2. Soil drench

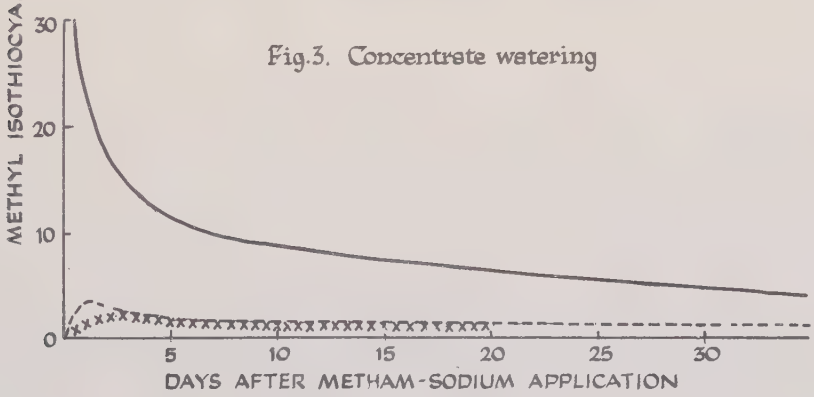

Fig.3. Concentrate watering

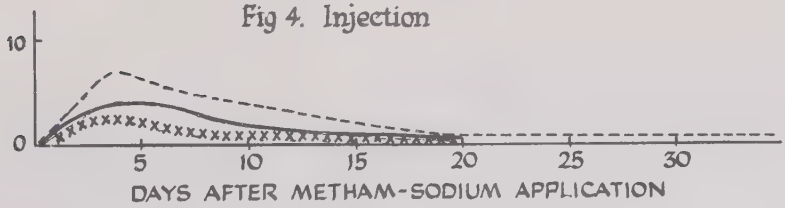

Fig 4. Injection

NABAM, NEW FORMULATION AND NOVEL USES

by D. Tyson
Pan Britannica Industries Ltd.

Summary

Previous work with nabam as a 19% aqueous solution is reviewed, and the introduction of a new formulation as a 93% soluble powder (code name A.40) is discussed.

The control of Cladosporium fulvum on glasshouse tomatoes by the use of a mixture of A.40 and zinc sulphate has been shown to be as good as the approved and established mixture of liquid nabam and zinc sulphate.

Positive action was shown against certain soil pathogenic fungi such as Pythium, Phytophthora spp., and Plasmodiophora brassicae, by the use of A.40 as a soil drench prior to sowing or planting, or as a powder dressing of seed.

The Brown Root Rot complex on glasshouse tomatoes was reduced and yields increased by the continuous watering of the established tomato plant roots with a solution of sixty parts A.40 to one million parts water. No phytotoxicity has been noted. The application of a mixture of A.40 and a liquid fertiliser is possible without any loss of efficiency if applied within one hour of mixing.

Introduction

Hester in 1943 was granted a U.S. patent protecting the use of disodium ethylenebisdithiocarbamate (nabam) as a fungicide, but this compound proved too unstable and too soluble for use on foliage until Heuberger et al. (1947) stabilized it with a mixture of lime and zinc sulphate, a mixture patented in the U.S A. by Heuberger in 1948. Later work showed that the addition of lime was not necessary. In the U.S A. the nabam/zinc sulphate tank mix has been widely used for many years for the control of such pathogenic fungi as Alternaria solani, Helminthosporium turcicum, Phytophthora infestans and Stemphylium solani.

The first work on the control of a foliar disease by means of a nabam/zinc sulphate tank mix in the U.K. was reported by Beaumont (1954). He showed that the control of Cladosporium fulvum on tomatoes with a mixture of liquid nabam containing 19% disodium ethylene-bisdithiocarbamate with zinc sulphate (22.7% zinc) was better than with other materials tested, and he also reported that this tank mix was the only spray to remain efficacious in severe epidemics. Wiggell (1958) continued this work, and his results were in close agreement with Beaumont's findings. At present in the U.K. the nabam/zinc sulphate tank mix is used almost exclusively for control of Cladosporium fulvum on tomatoes under glass. For field crop diseases zineb, the zinc salt of the parent acid, is preferred.

For many years both European and American research workers have been examining the possibility of using nabam with its wide fungicidal spectrum for the control of soil-borne parasitic fungi.

Zentmeyer (1955) reported that nabam was fungicidal to Phytophthora cinnamoni at 25 p.p.m. of soil and to Verticillium alboatrum at 250 p.p.m. He also stated that when nabam was drenched onto the soil the fungicidal effect was more than one hundred times greater than that of zineb. The effects of nabam on soil fungi and bacteria are described by Strecker (1957) and Zanardi (1957).

Messiaen and Lafon (1957) recommended the addition of 0.5% or 1% nabam to hot water for the treatment of maize seed against Gibberella zeae. Sinclair (1957) assessed nabam as a promising chemical for control of Rhizoctonia solani when applied to the soil at sowing time. Whitehead and Brown (1957) showed nabam to give control of Rhizoctonia solani, Pythium and Fusarium spp. when applied to the seed drill.

Grossman (1957) could show no chemotherapeutic effect when a nabam solution was applied direct to roots of tomato plants before or after infection with Fusarium oxysporum f. lycopersici, Alternaria solani and Phytophthora infestans. Very slight root damage was noticed with a concentration of 20 p.p.m., and slight damage with 100 p.p.m. Domsch (1958, 1959) in a series of tests with various soil fungicides showed that nabam gave good results against Pythium spp. and Rhizoctonia solani, although its fungicidal persistence was short. The ED50 value for Pythium was reached in 15 days after application at 100 p.p.m. of soil. In comparison the ED50 value of zineb at 800 p.p.m. was not reached until 80 days after application. He suggested that naban increased the number of soil bacteria and actinomycetes, and markedly reduced the fungal population.

He (1960) further reported that nabam at 100 p.p.m. greatly altered the microflora balance of the compost. Amongst the fungi controlled were Verticillium dahliae var. zonatum, Cephalotrichum medium, Mortierella exigua, Volutella ciliata. Tolerance was shown by three Chaetomium spp. and Penicillium nigricans. Trichoderma viride was reduced by fifty per cent. Corden and Young (1960) reported nabam to be more effective than metham-Na or zineb against Fusarium oxysporum f. cubense chlamydospores when mixed with artificially air-dried soil.

In England studies on nabam as a soil fungicide were started by Wiggell and others on the control of certain root rots on glasshouse tomatoes by constant watering with a weak solution of liquid nabam (300 p.p.m. of the 19% material) after planting in final quarters. This weak solution was used in place of water for normal watering procedure and gave favourable results (Wiggell, 1961).

Up to 1960 all the commercial work involving the use of nabam had been carried out with the 19% aqueous solution of nabam. It had not proved possible to formulate a stable powder form, although the advantages of such a material were well realised. In 1960 trial quantities of a powder form containing 93% disodium ethylene-bisdithiocarbamate became available. This material (code name A.40) is completely soluble and reacts with metal salts in aqueous solution as does the standard liquid formulation of nabam.

Experimental

The aims of the trial work were: (a) To ascertain whether the control of Cladosporium fulvum was as good with powder nabam/zinc sulphate as with the established liquid nabam/zinc sulphate; (b) To provide data on the control of soil-borne parasitic fungi prior to sowing or planting; (c) To determine the effect on the plant and yield of constant watering of tomato plants with a nabam solution using A.40 at 60 p.p.m., and to examine the possibility of applying to the soil a combined liquid nutrient and nabam solution; (d) To determine the effect on Brown Root Rot. Some doubt exists as to the exact nature of this disease which is widespread and found in most glasshouse soils where tomatoes are grown.

Results

1) Nabam as a foliar spray for control of Cladosporium fulvum on tomatoes

Materials:-

Liquid nabam:- disodium ethylene-bisdithiocarbamate as a 22% solution plus zinc sulphate (22.7% zinc). Mixed at the rate of 1 gallon liquid nabam plus 2 lb zinc sulphate in 160 gallons water.

A.40:- disodium ethylene-bisdithiocarbamate as a 93% soluble powder plus zinc sulphate (22.7% zinc). Mixed at the rate of $2\frac{3}{4}$ lb A.40 plus 2 lb zinc sulphate in 160 gallons water.

Both materials applied as a high volume spray at 200-300 gallons per acre.

Key for Tomato Leaf Mould

(Developed by the Disease Assessment Committee of the Conference of Advisory Plant Pathologists.)

Per cent

0.1 Lesions found with difficulty, and on less than one plant in fifty.

1 Lesions on most plants, but only on a few leaves.

5 Lesions on every plant, and on most leaves except the young ones, but only about two to ten spots per leaf.

10 All except the youngest leaves affected, with ten to fifty spots per leaf.

25 All except the youngest leaves affected, but with about three-quarters of the leaf area green although lowest leaves may be severely attacked.

50 All leaves affected. Most of the middle leaves show only half their area green.

75 All leaves affected. Most of the middle leaves show only one-quarter of the leaf green, giving a grey appearance to the crop as a whole.

90 Very little green visible on middle and lower leaves, but youngest leaves show green.

100 All leaves completely covered with lesions.

Leaf Mould Assessment

Site	Material	Date of examination			
		July 12	July 26	Aug. 9	Aug.23
Yorkshire (I) (sprayed June 29 July 12 July 26 Aug. 9 and Aug. 23.)	Liquid nabam	0	0.5	0.1	0.1
	A.40	0	0.5	0.1	0.1

Site	Material	Date of examination			
		June 2	June 15	June 29	July 7
Yorkshire (II) (sprayed June 2 June 15 June 29 and July 13)	Liquid nabam	1.5	3.0	2.0	1.0
	A.40	1.8	3.5	2.5	1.0

Site	Material	Date of examination			
		June 6	June 20	July 7	July 21
Lancashire (I) (sprayed June 7 June 21 July 7 and July 21)	Liquid nabam	1.8	4.0	1.5	1.3
	A.40	1.9	3.8	1.3	1.4

Site	Date of examination				
	Material	June 6	June 20	July 6	July 20
Lancashire (II) (sprayed June 6 June 20 July 6 and July 20)	Liquid nabam	0	0	0.5	0.5
	A.40	0	0	0.5	0.5

Site	Material	Date of examination					
		May 5	May 22	June 6	June 20	July 7	July 18
Lea Valley (sprayed May 9 May 23 June 6 June 20 July 4 and July 18)	Liquid nabam	1.0	1.0	0.5	1.0	1.0	1.0
	A.40	1.0	1.0	0.8	0.8	0.5	0.5

2) Nabam as a soil fungicide

a) Control of damping-off diseases outdoors

The materials used were a commercial seed dressing containing captan, applied dry to seed, and A.40 applied at $\frac{1}{8}$ oz. in 1 gallon of water per 30 ft. of seed drill after sowing.

The layout of 4 replicates of each treatment and untreated control, on a gravel clay site which had been market garden for thirty years. Peas of the variety Onward were sown on 2/3/1961 and the weather, from sowing to germination, was wet and cold. The mean number of emerged codlings per plot were: A.40, 74.25; captan seed dressing, 72.00; untreated, 60.25, with a significant difference of 11.29 (P = 0.05).

b) Control of Club root (Plasmodiophora brassicae Woron) on cabbage

The materials used were: A.40 dip, 2 oz in 1 pint water as a preplanting dip; A.40 drench, 4 oz in 100 gallons water. 10 fl. oz of this dilute applied per plant upon planting out; Calomel, 4% commercial preparation used 1 lb in 6.6 fl. oz water, as a preplanting dip.

The layout of randomized plots, 5 replicates of each treatment and untreated control, with 16 plants per plot, on a site with a previous history of club root infection.

Cabbage (Primo) was planted 17/5/61, and the weather during growing period was hot and dry.

The results are assembled in Table 1.

Table 1 Control of club root

Treatment	Mean weight of cut heads of cabbage (oz)	Mean number of infected roots per plot	Notes
A.40 dip	4.18	0.2	Severe scorch noted on roots
A.40 drench	33.19	0	
4% Calomel	25.01	0	Slight scorch noted on young roots
Untreated	21.90	6.4	
	SD (p=0.05)= 6.02 oz	The root infection was in no case more than slight clubbing of laterals	

c) Control of damping-off diseases under glass

TRIAL I

The materials used were: A.40a, 1 oz in 12 gallons water applied to soil as drench before sowing; A.40b, 1 oz in 6 gallons water applied to soil as drench before sowing; A.40 seed dressing, 1 oz in $1\frac{1}{2}$ gallons water, seeds dipped therein before sowing; Cheshunt Compound, a commercial preparation used at 1 oz in 1 gallon water, applied to soil before sowing and again after germination. The layout was of randomized plots, 4 replicates of each treatment and untreated control. Cress (Fine Curled), sown 16/6/1961 at a rate of 180 seeds per replicate, in soil infested with Pythium and Phytophthora spp. The results are given in Table 2.

Table 2 Control of damping-off

Treatment	Mean number of seedlings per plot 8 days after emergence	Notes
A.40a Soil drench	125.75	
A.40b Soil drench	111.75	Slight phytotoxicity noted
A.40 Seed dressing	79.00	Phytotoxicity noted
Cheshunt Compound	159.25	
Untreated	88.00	
	SD(p=0.05) 37.25	

TRIAL II

In this trial the materials tested were: A.40a, 1 oz in 12 gallons water, applied as a soil drench before sowing; A.40e, 1 oz in 18 gallons water, applied as a soil drench before sowing; A.40f, 1 oz in 24 gallons water, applied as a soil drench before sowing, and A.40 seed dressing, seeds dressed with dry powder.

The layout of randomized plots, 4 replicates of each treatment and untreated control. Lettuce (All the Year Round) were sown on 3/7/1961 at the rate of 150 seeds per plot on soil infested with Pythium and Phytophthora spp. The results are given in Table 3.

Table 3 Control of damping-off

Treatment	Mean number of emerged seedlings per plot 10 days after emergence	Notes
A.40a Soil drench	82.75	Phytotoxicity and growth retardation noted
A.40e Soil drench	87.00	Growth retardation noted
A.40f Soil drench	126.50	
A.40 Seed dressing	136.00	
Untreated	91.75	
	SD(p=0.05) 33.80	

TRIAL III

The materials used were: A.40e, 1 oz in 18 gallons water, applied as a soil drench before sowing; A.40 seed dressing, seeds dressed with dry powder; Cheshunt Compound, a commercial preparation, 1 oz in 1 gallon water, applied to soil before sowing and after germination.

The layout of randomized plots, 4 replicates of each treatment and untreated control, planted with tomato (Moneymaker), sown 20/7/1961 at a rate of 10 seeds per plot on soil infested with Pythium and Phytophthora spp. Table 4 gives the results.

Table 4 Control of damping-off

Treatment	Mean number of emerged seedlings per plot 10 days after emergence
A.40e Soil drench	7.25
A.40 Seed dressing	7.00
Cheshunt Compound	7.50
Untreated	3.05
	SD(p=0.05) 2.81

3) Nabam as a soil fungicide on growing crops

Previous work had shown that a continuous application to growing tomato roots of a 300 p.p.m. solution of nabam, as a 19% preparation, had not resulted in phytotoxic effects.

Method:-

A.40, was made into a stock solution of 2 oz to 1 gallon water. This was added to the irrigation water through a dilutor to give a concentration of 60 p.p.m. This weak solution of nabam was used in place of water for the normal crop watering procedure.

To ensure that this application would be suitable for cultural practices entailing constant inclusion of liquid fertilisers with the irrigation water the compatibility of the nabam solution with liquid fertilisers was examined.

a) Compatibility of A.40 solution with a commercial liquid fertiliser

A.40 added to liquid fertiliser as 1.25% or 2.5% solutions.

Liquid Fertiliser mixed with A.40 (w/v analysis) N : P_2O_5 : K_2O	Rate of Decomposition of A.40 (% loss of nabam per hour)	
	1.25% solution	2.5% solution
3 6 12	0.40	0.32
6 0 7	0.43	0.30
6 6 7	0.47	0.32
24 6 0	0.52	0.35
Tap water pH 7.0	0.03	0.02

b) Treatment of seedlings and young plants

Tomato seeds were sown in unsterilised soil taken from a glasshouse with a long history of tomato growing. After germination half of the number of seedlings were watered as needed with a 60 p.p.m. aqueous solution A.40. The other half received plain water.

At the time of pricking out into pots the seedlings showed:-

Treatment	Average length of seed leaf	Average length of 1st true leaf
A.40 drench	40 mm.	20 mm.
Plain water	28 mm.	15 mm.

At the time of appearance of the 1st truss the plants showed:-

Treatment	Average length of the leaf immediately below the 1st truss	Average height of plant
A.40 drench	156 mm.	227 mm.
Plain water	131 mm.	203 mm.

c) Treatment of established tomato plants

The application of A.40 as a 60 p.p.m. solution began with the initial ball watering. The same dilution was applied throughout the life of the crop. The amount per plant was the same as the amount of plain water given to each untreated plant.

Assessment of Brown Root Rot on Tomato Roots

This was based upon the following:-

0 = All white root.

1 = Slight corky lesions on some roots.

2 = Corky lesions with some brown rot on a few roots.

3 = 50% roots showing severe corky lesions with brown rot on some roots.

4 = Brown rot on 50% roots with little clean white root to be seen.

5 = All roots diseased.

Performance of nabam

Site L

Soil type:-	Peat soil.
Soil sterilisation prior to planting:-	Carbon disulphide.
Planting data:-	Tomato (Moneymaker) on 23/4/61
Treatment:-	200 plants receiving A.40 solution, 200 plants receiving plain water.

Treatment	Height of plant at 25/7/61	Total weight of crop per plant (21/9/61)	Brown Root Rot assessment on 21/9/61
A.40 solution	73 in.	83 oz.	1.6
None	62½ in.	65 oz.	3.9

Site B

Soil type:- Loam with high clay fraction.

Soil sterilisation prior to planting:- None

Planting data:- Tomato (Ailsa Craig) on 4/4/61

Treatment:- 380 plants receiving A.40 solution
350 plants receiving plain water.

Treatment	Height of plant at 25/7/61	Total weight of crop per plant (25/9/61)	Brown Root Rot assessment on 25/9/61
A.40 solution	87 in.	80½ oz.	2.1
None	81 in.	72 oz.	4.2

Site C

Soil type:- Gravel Loam with gravel and medium clay fraction

Soil sterilisation prior to planting:- None

Planting data:- Tomato (Moneymaker) on 12/4/61

Treatment:- 400 plants receiving A.40 solution
400 plants receiving plain water

Treatment	Height of plant at 22/7/61	Total weight of crop per plant (18/9/61)	Brown Root Rot assessment on 18/9/61
A.40 solution	77 in.	79½ oz.	1.8
None	69 in.	69 oz.	3.8

Site E

Soil type:- Silt loam

Soil sterilisation prior to planting:- None

Planting data:- Tomato (Ware Cross) on 17/4/61

Treatment:- 300 plants receiving A.40 solution
300 plants receiving plain water.

Treatment	Height of plant at 22/7/61	Total weight of crop per plant (22/10/61)	Brown Root Rot assessment on 2/10/61
A.40 solution	72½ in.	97 oz.	1.2
None	65 in.	80½ oz.	3.5

Site W

Soil type:- Sandy loam

Soil sterilisation prior to planting:- D.D. injection

Planting data:- Tomato (Ware Cross) on 2/3/61.

Treatment:- 200 plants receiving A.40 solution
200 plants receiving plain water (This area had received 2% formaldehyde before planting as well as the D.D.)

Treatment	Height of plant at 29/7/61	Total weight of crop per plant (28/9/61)	Brown Root Rot assessment on 28/9/61
A.40 solution	83½ in.	98 oz.	1.8
None	79 in.	90 oz.	3.6

Discussion of Results

1) Nabam as a foliar fungicide

The trial work reported shows that the tank mix of A.40 and zinc sulphate gives equally good control of Cladosporium fulvum as does the established tank mix, when both were applied as a high volume spray.

It proved easier to mix the A.40 and the zinc sulphate together than it did to mix the liquid nabam and zinc sulphate; in both cases the procedure was: - Mix the nabam formulation with one third of the required volume of water, stir in the zinc sulphate and finally mix in the remaining two thirds of water.

The use of A 40 did present a slight disadvantage at mixing time, as the powder was found to be slightly irritant to the nose and throat passages. This could be minimised by careful handling of the powder. During the spraying of tomato crops there was no difference in the effect of A.40 or liquid nabam mixtures upon the nose and throat passages of the operator.

Laboratory results indicate that the present compatibility of liquid nabam and zinc sulphate with 25% miscible D.D.T , kelthane, lindane, magnesium sulphate, malathion and nicotine as a joint high volume spray will also apply to A.40.

2) Nabam as a soil fungicide prior to planting or sowing

The result given of the use of A.40 as a chemical control of pre-emergence damping-off indicates that the use of A.40 gives a significant increase in the number of emerged pea seedlings compared with the untreated plots. The other chemical used, captan, also gave a significant control of pre-emergence damping-off.

A.40 also gave significant control of club root (see Table 1) on cabbage when applied as a soil drench to the base of the seedling immediately after planting. The dipping of seedlings into a strong solution of A.40 led to scorching of the roots with a corresponding drop in weight of the cabbage cut from that treatment. The dipping of seedlings into one of the standard recommended methods of control, namely 4% calomel paste, also showed some scorch on the roots and, while this method did control the attack of club root, the yield was not significantly better than that from the untreated areas. This was possibly due to the very hot, dry weather which continued throughout the growing life of the cabbage, and which undoubtedly reduced the degree of infection by club root on the trial area to the low level reported. The adverse weather together with lack of irrigation affected other trials containing various methods of application of A.40 as a possible control of club root to the point where no significant data was obtained at all. One method of application which holds promise is the rotovation into the affected soil of A.40 7 to 14 days before planting or sowing crucifers.

A drench of A.40 at various dilutions on soil infected with Pythium and Phytophthora spp. before sowing lettuce, cress and tomato, showed a significant increase of emerged seedlings over the untreated sowings (see Tables 2, 3 and 4). Also the dressing of tomato or lettuce seed with the dry A.40 gave a significant increase over the untreated seed. Cheshunt Compound, used in two of the reported trials, was not significantly better than A.40. Cress was not damaged by a pre-sowing drench of 1 oz A.40 dissolved in 12 gallons of water, whereas lettuce growth was definitely retarded and germination impaired by such a solution and by the 1 oz A.40 in 18 gallons. Good results were obtained with 1 oz A.40 in 24 gallons and with a seed dressing of the dry A.40 before sowing. Tomato seedlings tolerated 1 oz A.40 in 18 gallons, good results being obtained with this method and with the dressing of seed with dry A.40. The coating of cress seeds with a strong dilution of A.40 before sowing resulted in fewer germ-

inated seedlings.

It can therefore be seen that the treatment of seeds with the dry A.40 before sowing is promising although more species remain to be treated before this remark can be taken as a general conclusion. The drenching of soil before sowing, while proving satisfactory, showed that the tolerance of certain seeds to a soil treated just before sowing with different strengths of A.40 in aqueous solution varies. As the half-life of nabam is comparatively short it may prove more practical to drench the soil 7 days before sowing thus obtaining control of the pathogens without phytotoxicity.

3) Nabam as a soil fungicide on growing crops

In the U.K. generally the glasshouse tomato is being grown on soils with a long history of tomato culture, and it is therefore not surprising that soil diseases cause a loss of potential crop every year on most holdings even where the partial sterilisation of the soil is practised. One disease which is proving troublesome is the Brown Root Rot complex of which the exact nature is not yet known. Ebben (1949) described the disease as consisting of root lesions varying in colour from pale to dark brown, with the root cortex either split and flaking-off or swollen and "corky" in appearance, girdling the root. On some lesions the whole root cortex rots away leaving a brittle vascular cylinder. The cortex at the base of the stem and below may also be affected. This description agrees with that of McKay (1949), who describes it as Tomato Root Rot, and with that of Noordam, Termohlen and Thung (1957). The causative agent of Brown Root Rot has not yet been agreed upon but McKay (1949) gives Colletotrichum atramentarium as the agent. Work carried out at Kvithamar Experimental Station was reported by Roll-Hansen (1952) who stated that the most prevalent fungus associated with Brown Root Rot was Cylindrocarpon radicicola, and that fungal damage was secondary to some other cause as yet not elucidated. Williams and Ebben (1950-1953) working at Cheshunt Experimental Research Station found Fusarium spp, Colletotrichum atramentarium, Chaetomium cochliodes, Volutella ciliata, Petriella asymetrica and a sterile grey fungus "K" on the root surface and in the affected tissue. They suggested that more than one fungus might well be the causative agent, as the species present on diseased roots varied. Their opinion was that Colletotrichum, Chaetomium, Volutella and Fusarium were the more likely agents. Noordam, Termohlen and Thung (1957) found that a sterile grey fungus taken from diseased roots would, when inoculated into tomato soils, cause symptoms on tomato roots similar to Brown Root Rot. Ebben (1959) found Cephalosporium spp. on the affected root surface and tissue. He (1960) also observed no difference in bacterial population of the rhizosphere of root surface which could be correlated with the development of Brown Root Rot.

Steaming the soil or treatment with metham-Na gives good control of this disease, but for many growers steam is too costly or not available, or the cropping schedule does not allow the use of metham-Na with its necessary 10 week delay between treatment and replanting. In such cases the control, or reduction in severity, of Brown Root Rot by the use of a

non-phytotoxic chemical applied to the growing crop would be welcome. The treatments given in this paper indicate that A.40, when mixed at 60 p.p.m. in the irrigation water and applied continuously throughout the life of the established tomato plant, gives a reduction in amount of Brown Root Rot and an increase in yield.

No phytotoxicity is caused by A.40 at 60 p.p.m. Stronger dilutions of 120 p.p.m. have checked young tomato plants and turned lower leaves bright yellow. Although the plants eventually grew away from this check they never regained their normal growth. A.40 at 240 p.p.m. caused the same effect - but it was more severe in appearance and longer in duration. One application of one quart of A.40 at 400 p.p.m. proved slightly damaging to young roots of mature tomato plants. Tomato leaves sprayed with A.40 at 60 p.p.m. showed no signs of damage or retardation even where they had already received routine sprays of liquid nabam and zinc sulphate for control of Cladosporium fulvum.

The reduction in amount of Brown Root Rot and increase in crop yield, from 9 to 25 per cent, following treatment with A.40 throughout the season was found on various soil types:- sandy loams, clay loams, peat soils etc. and this continuous application in no way affected the pH of any soil type when measured against the untreated areas. On one soil which is normally covered with blue-green algae during the life of the tomato plants, the area treated with A.40 had no such growth.

On many holdings it is the practice to apply liquid nutrients to the tomato crop constantly throughout the season, and here A.40 has the advantage of compatibility with a commercial liquid fertiliser. It has been shown that the breakdown rate of A.40 in tap water is very slight but when mixed with a commercial liquid fertiliser this breakdown rate is accelerated. However, the loss of A.40 is very slight in the first hour after mixing, and no loss of efficiency resulted when a combined A.40, liquid fertiliser is applied within that hour. An added advantage to users of automatic ground or low level irrigation systems is the complete solubility of A.40 and consequent avoidance of nozzle blocking or settling out of the material in the dilutor.

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Contribution by Dr. L. Broadbent

Despite good results which some soil sterilants give, we need to know a lot more about their mode of action and why they sometimes fail. It may interest you to know that we at the Glasshouse Crops Research Institute intend to do more work in the near future on the distribution and activity of soil micro-organisms. I think it is essential that we should know more about the biology of nematodes and all the organisms we are trying to control by soil sterilisation.

Q. Dr. M. Cohen

Mr. Tyson did not make it clear whether he was watering on nabam plus zinc sulphate in his tomato experiment or nabam alone. Would he confirm that it was nabam since zinc toxicity might be a problem to be faced if the mixture was being used?

A. Mr. D. Tyson

The soil watering was carried out with nabam alone.

Q. Mr. J. R. L. Stainton

Can Dr. Flipse explain why it is necessary to employ injection methods for metham-sodium into hyacinth seed beds rather than using the drench method.

A. Dr. L. Flipse

No, we have no good explanation for this. We think that with the drench method the penetration will not go deep enough. Hyacinths are planted rather deeply and they root very deeply. It is only because of our experience over the course of four seasons that we have come to this conclusion. Every year we have observed that the injection method gave superior results to the drench method. A supplementary treatment with formaldehyde is also necessary.

Q. Dr. L. Broadbent

One of the most interesting practices that is being developed in the U. K. at the moment is that of watering dilute fungicides on the soil at frequent intervals during the growth of the crop. Is this done on the Continent?

A. Dr. W. Madel

This practice is widespread in horticulture in Germany. Zineb and sometimes captan are used at intervals of 1 to 2 weeks.

Comment by Dr. L. Flipse

As far as I know the practice of application of fungicides during growth periods as a soil drench is not used in Holland.

Q. Dr. L. Broadbent

Can the people in the U.K. who are using this method comment on whether it is expensive?

Contribution from Mr. D. Wiggell

Nabam costings at the rate used in trials worked out at about £90 per acre. Dilutors at present in use for the application of fungicides to tomato plants are inaccurate and could possibly lead to applications of the fungicide at rates which would be phytotoxic to the plants. At the moment this somewhat limits the use of the method in commercial practice.

Contribution from Mr. D. Tyson

We have found costs to work out at about £60 per acre but they could go up with an early crop. I would also like to confirm the fact that an efficient dilutor is necessary and phytotoxic effects may be felt if tomato plants are grown in soil badly infested with Root-knot Eelworm.

Q. Mr. D. G. Fenemore

Apart from the special consideration of Root-knot Eelworm, is metham-sodium recommended in Holland for use prior to growing cucumbers?

A. Dr. L. Flipse

Two reasons why we cannot recommend metham-sodium on cucumbers.
(a) It has been the experience for many years that other fumigants (D.D. and Nemagon) are superior. Root-knot eelworm in cucumber is a special problem, much more difficult than in tomatoes, I think because of much deeper rooting and much heavier infestation of Root-knot eelworm at a greater depth in the soil.
(b) Another reason is that cucumbers in glasshouses are grown in soil mixture of very high content of organic matter into which probably the chemical is too much absorbed. In fact we have seen several times failure from tests with metham-sodium on cucumber.

Q. Dr. L. Broadbent, Chairman

We have heard this afternoon something about the use of metham-sodium outside glasshouses on the Continent. Do nurserymen and foresters use it in this country on their seed beds?

A. Mr. D. Tyson

I can confirm that there is a recommendation on several product labels regarding this use. The crop must be a valuable one to justify the use of a high cost chemical. It is used in the Channel Islands at very low rates - 25/30 gallons/acre - for valuable tomato crops and on potato crops for partial control of potato root eelworm.

Q. Dr. L. Broadbent

Why does such good control of the nematodes on potatoes have such poor effect on yield? What is the effect of Heterodera rostochiensis on tuber formation? Does the number of tubers or does their size decrease? What is the effect of the nematodes on the plant?

A. Dr. J. E. Peachey

I think the problem of Heterodera rostochiensis is that we are dealing with a parasite which has a rather close relationship with the host. Very small infestations can do a great deal of damage. It is a question of the competition curve, and we come to a certain point with heavy infestations where reduction in population must be very extensive to show any effect on yield. Work in Scotland points to this conclusion. As regards tuber formation, the interesting thing about eelworm attack is that when you do get a crop failure, there are hardly any tubers at all. It is fair to say that under severe conditions, both tuber size and number decrease; under moderate conditions the tuber number decreases and with good control there is an improvement in both number and size but mainly in size.

Q. Dr. L. Broadbent, Chairman.

Does the nematode excrete any chemical which has an effect on tuber formation?

A. Dr. J. E. Peachey

Substantial reduction in root growth follows from heavy infestations and presumably the roots cannot support tuber formation.

Q. Dr. L Broadbent, Chairman.

In Mr. Tyson's paper, he refers to the control of Cladosporium fulvum by A.40 comparing it with liquid nabam. I agree that all these figures show that the level of leaf mould is about the same with the two. Is there any evidence that either of them caused any decrease in disease?

A. Mr. D. Tyson

As liquid nabam plus zinc sulphate is an approved control of Leaf Mould it was not felt necessary to do more than compare powder nabam, A.40, plus zinc sulphate with it.

However, considerable evidence is available to show that either liquid or powder nabam plus zinc sulphate does give a significant control of Leaf Mould when compared to untreated plots.

Q. Dr. Swarbrick

The pictures which Mr. Read showed of the damage to tomato plants caused by the vapour of sodium N methyl dithiocarbamate approaches that caused by certain growth regulating substances. This may help to explain two cases of plants sent to me during the summer. By the time I got them the leaves were nearly dead but there was epinasty and malformed tomatoes, although the information was that no growth regulators had been used.

My question is, is much of this material being used in practice, if so are growers aware of the damage that may be caused by even small amounts of the vapour of this substance?

A. Mr. W.H.Read

These serious phytotoxic effects were prevalent in the first year of its use but now, by conforming to instructions, these cases of acute damage are rare. A large quantity of metham-sodium is being used in glass-houses in this country.

Comment from Dr. Swarbrick

But the risk is there still?

A. Mr. W.H.Read

Yes, there is slight risk. We are trying to find out what is responsible.

Q. Mr. R.J.Collins

Could Dr. Peachey comment on recent reports on the use of sugar to control eelworm?

A. Dr. J.E.Peachey

The doses required and costs involved to put enough sugar in the soil would be quite impossible from the economic point of view, and certain preliminary experiments by Mr. Rao, suggest that the phytotoxic effect is great. The idea is a novel one but the dosages involved and problem of mixing would be fantastic.

THE ANALYSIS OF PESTICIDE RESIDUES IN FOODSTUFFS: A REVIEW OF RECENT DEVELOPMENTS IN ANALYTICAL METHODS

by H. Egan and E. Q. Laws
D.S.I.R., Laboratory of the Government Chemist

When pesticides are applied to growing crops, the active ingredient of the preparation used will either remain on the surface of the plant or be absorbed into the plant system, depending on the nature of the pesticide, the manner in which it is formulated and the nature of the plant. If a compound is taken into the plant circulation system and is subsequently translocated within the plant, it is said to be systemic. The main factor which determines whether a substance is systemic or not is whether it is itself water soluble or is easily converted to a water soluble form by oxidation.

In certain plants absorption of a non-systemic compound may take place in parts of the plant, without subsequent distribution throughout the plant. Systemic compounds may undergo enzymic oxidation and breakdown to other products within the plant, in some cases with the production of substances which are more toxic to mammals than the original pesticide. When pesticides are applied to living animals similar considerations apply but the results are not always the same as with plants owing to the greater complexity of the enzyme systems of the animal organism. Thus the same pesticide, gamma-BHC, is partially stored by rats but appears to be rapidly metabolised by rabbits (Jondorf et al. 1955). In general, substances which tend to persist are preferentially soluble in non-aqueous solvents and concentrate in the fatty tissues.

In any method of analysis, the above considerations must be taken into account. This paper does not set out to review methods of analysis as such: reviews of this kind are available elsewhere (Chilwell and Hartley, 1961; Westlake, 1961). The present purpose is to outline the problems which face the analyst examining foodstuffs for pesticide residues, to describe the various approaches which have been made to the problem and to say something of the present and future possibilities in the analytical field.

The essential problem presented to the analytical chemist in the detection and determination of pesticide residues in foodstuffs is that of specifically and accurately measuring a few micrograms of a complex, usually organic chemical in the presence of a much larger quantity of many other complex organic species. The general problem is presented in its most acute form to the public analyst (in the widest sense of the phrase) who, unlike his industrial colleague concerned with the company's field or other trials, may have no idea at all as to which, if any, of the possible residues may be present.

Virtually all methods for residue analysis are made up of three distinct stages: (a), extraction of the residue from the foodstuff into a suitable solvent; (b), the removal from this extract of co-extracted material which otherwise would interfere with the final method of determin-

ing the residue - the so-called 'clean-up' stage; and (c), the determination of the residue in the cleaned-up extract. The three stages usually follow the sequence above. Each stage presents its difficulties. Solvent extraction must be thorough but must nevertheless avoid the complication of intractable emulsion formation whereby although the residue is successfully separated from the food into the solvent, the solvent itself becomes inseparable from the sample. The choice of solvent may itself contribute to the clean-up process. The clean-up must not only remove interfering substances from the extract but it must not occasion losses of the extracted residue in the process. Specificity is a major difficulty in the final method of determination, even where the unknown residue is obtained as a simple solution: several complex pesticides may offer almost identical analytical characteristics and the fact that the unknown is in very dilute solution adds to the difficulty. Such difficulties are also illustrated by the fact that several technical pesticidal materials contain impurities of like composition, whilst others may give rise to metabolites which though of similar chemical constitution nevertheless have different toxicological properties. The isomers of hexachlorocyclohexane or of endosulfan, or the degradation of heptachlor to heptachlor epoxide and the oxidation products of phorate are examples.

In addition to the difficulties outlined above, the residue itself may be unstable (which may be all to the good so far as the consumer of the produce is concerned); the residue may be sensitive to change due to storage conditions or to circumstances arising from the analysis itself; and in all of these processes contributions to the final analytical result by the reagents themselves - the reagent blank - must be kept to a minimum, as must also losses of the residue during what may be a long series of laboratory operations.

Obviously the ideal approach to the problem of residue analysis would be to choose a highly sensitive and specific analytical method which will detect and measure a few micrograms of pesticide X in the presence of anything else. This is in fact the aim: more than this, the method should detect and measure pesticides X, Y, Z, ... in the presence of anything else. Here it can immediately be pointed out that in the search for such a method practically all the devices, old and new, known to the analytical chemist - and many of those known more particularly to his biologist, entomogist and other colleagues as well - have been investigated. The methods in the literature include the classical analytical techniques such as titrimetry or gravimetry; spectrophotometry in the ultra-violet, visible and infra-red regions; electrochemical methods including amperometric titrations, coulometry and polarography; column, paper and gas-liquid chromatography; and radio-chemical analysis. They also include bioassay methods and methods dependent on the selective action of enzyme preparations.

Methods can be roughly sub-divided into two kinds, specific and non-specific; in practice the division is not always a sharp one. Specific chemical methods often rely on a well-known property of one of the func-

tional groups of a molecule, such as a nitro group or a phenolic group, for the production of a measurable colour, and are in fact only specific in a relative sense. Again, the degree of specificity of such methods may depend as much on the extraction or clean-up processes as on the final method of analysis. The non-specific chemical methods also rely on preliminary extraction and clean-up stages but are dependent thereafter on the conversion of the residue to an inorganic form such as chloride, phosphate or sulphide, which is then measured by a suitable micro-method. It is particularly necessary in these methods to ensure that compounds of the element naturally present in the sample foodstuff do not interfere with the determination, a fairly straightforward matter for chlorine but less so for phosphorus or sulphur. It is also necessary to ensure that the reagents used are free from the element in question; this is a fairly easy matter for phosphorus but less easy for chlorine or sulphur.

When experimental samples, for example of a vegetable crop, are treated with known quantities of pesticide under controlled conditions, the results obtained by non-specific methods may provide very good evidence of the residue level present; but if the presence and nature of any residue is unknown, it is not possible to distinguish for example, which of the many organo-chlorine pesticides is present from a non-specific micro-chlorine determination. If the extraction and clean-up methods are properly chosen, the classical non-specific methods may be more precise (though perhaps less informative) than a specific chemical methods for synthetic pesticide residues. Successful design of the extraction and clean-up methods may go some way to introducing specificity into a general method; thus in the scheme devised by Laws and Webley (1961) for organo-phosphorus residues in vegetables the polar residues are first separated from the relatively non-polar residues by solvent partition and the non-polar compounds further divided into two groups according to their relative affinity in solution for an activated alumina column.

The critical stage in the analysis is frequently the clean-up. This usually depends on physical processes such as selective absorption from alumina, silicate or magnesia columns followed by elution of the residue with a solvent, or on partition of the residue and other interfering extractives between solvents as in the Jones and Riddick (1952) acetonitrile method. These solvent or column partition techniques require calibration for recovery, using known amounts of the pesticide in the presence of other constituents such as colours and waxes naturally present in the sample extract. There is an inherent difficulty in introducing a precisely known level of pesticide into a sample of plant or animal tissue in exactly the same form as it would occur as a result of field application: radiochemical methods have been used to monitor various alternative extraction methods (Klein, 1959).

Purely physical methods are also widely used for the final analytical stage, as where the residue itself possesses characteristic absorption properties in solution: the low ultra-violet region remains relatively unexplored in this connexion, due mainly to the difficulty of finding a suitable solvent transparent down to about 170 millimicrons. Infra-red

absorption curves are highly characteristic for individual compounds, but the sensitivity is much lower than the ultra-violet method.

The original aspiration of the residue analyst some ten or fifteen years ago was to devise an analytical system which resembled the old group analysis scheme, whereby an unknown residue could be isolated and by sequential tests its identity assigned to one or other of a number of groups of closely related substances and, ultimately by further eliminatory or confirmatory tests, assigned to a single pesticide. In the course of work to this end many useful general methods of chemical micro-analysis have been published but the more specific colorimetric methods, e.g. for DDT, (Report of DDT panel, 1960) parathion (Averell and Norris, 1948) BHC (Wood, 1960) or dieldrin (O'Donnell et al., 1955) are often preferred where the identity of the residue is not in question.

Some of the specific methods are tedious and require special training on the part of the analyst. In recent years a prospect has been offered that some of the newer physical techniques such as gas-liquid or paper chromatography and infra-red spectrography, can give qualitative and quantitative information for residue levels in a more direct way. Indeed, it is to some extent true that gas-liquid chromatography can dispense with the need for tedious clean-up methods (Goodwin et al., 1961). Investigations into the application of all of these methods are the subject of current work, both in industrial and government laboratories.

Gas-liquid chromatography is capable of very high sensitivity towards certain organo-chlorine compounds if an electron capture detection system is used: at the same time the response of much of the interfering co-extracted material from certain plant tissues is suppressed, so that again the clean-up problem is considerably simplified. Using a conventional argon detector, residues of a few micrograms can be separated, passed through a combustion furnace and the chloride produced measured micro-coulometrically, as in the equipment now commercially available in the United States which offers a complete screening test for several organo-chlorine residues at the 0.1 p.p.m. in less than an hour (Coulson et al., 1960).

Paper chromatography has proved useful for the separation and identification of many organo-chlorine residues, if care is taken to standardise the assembly of the chromatographic tank and the techniques of applying and measuring the spots, 0.5 micrograms of individual compounds can readily be detected and quantities of from 2.5 to 15 micrograms measured with reasonable accuracy (Evans, in press). Conventional extraction and clean-up methods are used, although it may be possible to adapt some systems so that part of the normal clean-up stage is done on the chromatographic paper (Menn et al., 1960). Both infra-red spectrometry and gas-liquid chromatography offer the prospect of a direct examination of simple extracts without extensive clean-up; they have the advantage of being rapid methods and both offer some degree of specificity. Infra-red examination can be highly specific but in order to use the method for micrograms quantities special instrumentation is needed; as with gas-liquid chromatography, the general accessibility of the method is bound

up with the increasing commercial availability of suitable instruments.

The present day tendency seems to be to take advantage of the modern rapid instrumental techniques by choosing a combination of these such that together they fulfil the two principal requirements of high sensitivity and high specificity. Thus, gas-liquid chromatography may provide an initial degree of specificity first by eliminating interferences due to background material and then by separating individual pesticides or groups of pesticides; from the peak height or area a quantitative indication is also given but this may be improved by the use of sub-micro chemical or electrochemical methods, or by the infra-red examination of the fractions using micro-cells and a beam condenser system. Alternatively an independent confirmatory identification may be obtained by arranging for the effluent to be run on a paper chromatogram followed by identification and measurement of the apots.

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ANALYSIS OF SYNTHETIC ORGANIC PESTICIDES IN WATER BY CHROMATOGRAPHY

by E. Hindin and G. H. Dunstan
Washington State University

The growing use of synthetic organic chemicals for the control of pests on croplands, forests and marshes poses a potential threat to the health of the populace.

In 1960 one sixth of the cultivated land in the U.S.A. was treated with synthetic organic insecticides and herbicides. Projecting the usage on a world-wide scale the area treated is enormous. Run off water draining these treated areas picks up minute quantities of pesticides.

DDT and aldrin have been found in several rivers in the United States, rivers which serve as raw water supplies for dozens of communities. So far the quantities of pesticide found have been far below human toxicity levels (Tarzwell, 1959). Studies by the Communicable Disease Center of the U.S. Public Health Service reveal that these chemicals may have a cumulative impact on human beings (Hollis and McCallum, 1959). Pesticides in water have been blamed for about 40% of the fish mortality in the United States in 1960, according to a preliminary report by the United States Public Health Service (1961).

Clearly there is a need for a suitable method for the separation, identification and estimation of pesticides in surface and ground water. This is no easy matter because of the solubility of the chlorinated organic and many of the new organic phosphorus and sulphur pesticides. The maximum concentration of most pesticides is one part or less in a billion parts of water. A means of removing the pesticides from water must be employed. Adsorption on activated carbon, ion exchange resins, and solvent extraction have been used with some success. The pesticides may be removed from the adsorbing medium by an appropriate solvent. Further concentration may be required to facilitate the handling of the solvent pesticide extract.

A screening programme was adapted by the Sanitary Engineering Section at Washington State University last year for the separation, identification and estimation of chlorinated and organophosphorus micro quantities. The screening programme uses two paper chromatographic methods and an enzymatic method as a presumptive test and gas chromatographic analysis as a confirmatory test.

The presumptive screening programme consists of three distinct methods: (a) a qualitative and semi-quantitative method developed by Mitchell (1958) of the U.S. Food and Drug Administration and modified by Zweig and Painter (1960) at the University of California for chlorinated pesticides, (b) a paper chromatographic method of Mitchell (1960) for the identification of organophosphate pesticides, and (c) the quantitative enzymatic method for organophosphate and carbamate pesticides by Cook (1954)

Paper chromatographic methods:

The paper chromatographic method for the analysis of chlorinated organic pesticides uses an immobile-mobile solvent. The immobile solvent is 5% mineral oil in ethyl ether while the mobile solvent is 75% acetone in water. A chromogenic reagent is used consisting of silver nitrate in 2-phenoxyethanol, water, and acetone. The pesticides are delineated by exposure to strong short-wave ultra violet light when they appear as dark brown spots against a white background. The Rf values indicate the specific compound or class of compounds. The quantity of pesticide may be determined by visual comparison with known quantities of the respective pesticide or by the concentration to spot intensity-area relationship. This method has a number of limitations: (a) a number of chlorinated pesticides have the same or approximate Rf values, (b) some pesticides appear as streaks rather than spots, and (c) a number of chlorinated organic pesticides do not respond. The first two limitations can be minimized, however, there is no solution for the last one.

Paper chromatography for the detection of organophosphate insecticides is, in principle, similar to that used for the separation and identification of the chlorinated pesticides. The immobile solvent is 50% aqueous solution of NN-dimethyl formamide. An activating reagent consisting of bromine must be used. The chromogenic reagent consists of fluorescein and NN-dimethyl formamide in ethyl alcohol. The spots containing the pesticides are delineated under short-wave ultra violet light. Under ultra violet light the pesticides appear as a quench area that is dark purple against a greenish yellow background. If the organophosphate contains isomers a primary and secondary spot may appear. This method has a serious limitation: the contrast between the background and quenched areas begins to dissipate after 3 minutes and thus the method is not quantitative.

Enzymatic method

In order to obtain information of quantitative significance another approach must be taken. The quantities of organophosphate and carbamate pesticides are determined by an enzymatic method. The degree of deactivation of the enzyme acetyl-cholinesterase is indicative of the quantity of pesticide. It has been found that pseudo-oxidation of phosphorothioate and dithioates increases by a hundred fold the sensitivity of this method.

Gas chromatographic method

To confirm findings of the preliminary examination, extracts containing pesticides are subjected to gas chromatographic analysis. The pesticides are separated on a 6 ft. column of chromosorb W coated with Dow 11 high vacuum silicone grease at 250° C. Helium is used as the elutant. The pesticides are detected and quantitatively measured using thermo-conductivity cells. The electronic impulses are translated to a potentio-metric recorder. The thermal-conductivity cell detector can detect 0.5 mg. of

most pesticides. A hydrogen flame detector can increase sensitivity so that 1 microgram quantities of the respective pesticide can be detected.

Virtually all organic compounds containing pesticidal properties can be detected by gas chromatography. The detention time, the time taken for the component to pass through the column, is indicative of the specific organic pesticide. The area of the peak is also of quantitative significance.

In testing more than 90 synthetic organic pesticides we have found no two with the same Rf value and detention time value. The detention time value is of extreme importance in the identification of a specific pesticide whose Rf value is the same as that of another pesticide.

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Q. Mr. G. Ordish

Could Dr. Egan tell us if many samples of retail produce are examined?

A. Dr. H. Egan

Where such samples are examined by local authorities, details may be found in the annual reports of the Public Analysts. I would refer Mr. Ordish to these reports.

Q. Dr. J. T. Martin

The methods to which Dr. Egan has referred have been of the greatest value in the analysis of bulk samples of crops, e.g. to ascertain whether they conform to prescribed limits of residues. We are, however, badly in need of methods for determining the distribution of deposits over sprayed targets in work on the assessment of formulations or spraying methods. Here rapid methods, permitting large numbers of tests and suitable for deposits on small areas of plant surfaces, are required but so far are available for few of the spray chemicals.

A. Dr. H. Egan

Dr. Martin and his colleagues have developed some very elegant methods, which are both simple and sensitive, for assessing surface deposits of certain pesticide formulations; but I agree with him that a general method of wider applicability is desirable. I think the more complicated methods, used earlier by Winteringham to follow the metabolism of insecticides by insects, could be used. These methods require radioactive pesticides specially prepared for experimental trials.

NEW DEVELOPMENTS, INCLUDING NEW COMPOUNDS, NOVEL FORMULATIONS, OR NEW APPLICATION METHODS

Chairman: J. T. Martin
Long Ashton Research Station

INSECTICIDE GROUP

SYSTEMIC MIGRATION AND INSECTICIDAL ACTIVITY OF DIMETHOATE APPLIED ON TREE TRUNKS

by P. de Pietri-Tonelli*, A. Barontini* and G. Biondi**

Introduction

Defending plants from pests by endotherapic means is a problem which researchers have been trying to solve for a long time; but the answer has not yet been found despite the discovery of many organic insecticides with systemic properties. With the exception of the application to seeds and to the soil (methods which can be used only with vegetable crops), these substances are applied in relatively low concentration by the traditional technique of spraying or dusting the foliage of trees. The systemic properties are therefore exploited only partially for the active ingredient reaches a sufficient dosage only in certain parts of the leaves, in the less deep layers of the endocarp of the fruit, and in some portions of the bark of the branches and twigs (de Pietri-Tonelli and Barontini, 1961a, d). Because systemic insecticides migrate in plants mostly through the xylem, that is in an acropetal direction, a complete and rational utilization of the systemic properties would necessitate the application of the product to the roots or trunk.

Bypassing the application to the roots, which especially in the case of trees presents some disadvantages, let us examine the trunk application. The vast literature on this subject has been reviewed, among others, by Craighead and St. George (1938), Ripper (1957) and more recently, by Mitchell et al. (1960). The following are the advantages (partly already indicated by Jeppson et al. (1952) which might derive from the unusual application of the insecticide to the trunk of trees:

a) the possibility of distributing the product, by internal translocation, to every part of the plant, even to parts which can hardly be reached by other means;

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b) a simplification of the application technique and, possibly, independence of the availability of water and of certain meteorological conditions which are unfavourable with other means of application;
c) a reduction of antithesis between chemical and biological control owing to the absence of external toxic residues on large vegetable surfaces;
d) a reduction of the danger of contaminating cover crops and a lesser risk for the operator.

Although there are a few negative aspects or such that might be considered so (such as the difficulty in establishing easily the quantities of the product to apply to each plant; difficulties of the application technique; fear that the toxic residue variation may be unpredictable) we believe that the fundamental obstacle is created by the insufficient insecticidal activity of the products tested, or more often, by adverse effects on plants.

For the last few years, we have been making a basic study of the effects of the application of insecticides to the trunk and stem of plants (de Pietri-Tonelli and Barontini, 1961c; Santi, in prep.) and although we have achieved no conclusions of immediate application we are now reporting on the results of our work in the hope that they may contribute to the solution of the problem.

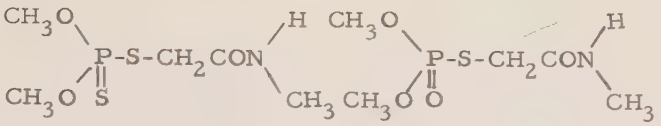
Materials and Methods

In this work we employed dimethoate (OO-dimethyl S-methylcarbamoylmethyl phosphorodithioate) and, in many experiments, also its P=O derivative (Table 1) which is its principal active metabolite (Santi and Pietri-Tonelli, 1959; 1960; Dauterman et al., 1959; 1960).

Unless otherwise indicated, the two products were used at 50% (w/v) in tri-n-butylphosphate (TBF) a solvent which permits highly concentrated solutions of the active ingredient. The solutions were applied to the plants by painting the tree trunk for varying widths (from 20 to 40 cm.) downward from the branches. Since it was necessary to carry out the experiments under natural conditions, with particular regard to the size of the plant, researches were conducted on trees in open fields, except in certain tests for phytotoxicity.

In order to determine the concentration of the product applied and of its possible metabolites having insecticidal properties, samples of leaves and fruit were bioassayed with Drosophila melanogaster at various intervals of time after application (de Pietri-Tonelli and Barontini, 1961b). In some cases P^{32}-labelled dimethoate (initial specific activity 1.43 mc/mM) was applied to the plants, which made it possible to determine, through radioanalysis and autoradiography the concentration of some of the transferred substances and the distribution of all of those which had in common the radioactive phosphorus of dimethoate (de Pietri-Tonelli and Barontini, 1961 a, c, d).

Table 1. Chemical, physical and biological properties of dimethoate and its P=O derivative.

	DIMETHOATE	P=O DERIVATIVE
Structural formula		
Water solubility	2.5 : 100	∞
Hydrolysis at 21° C Half life at pH 9 " " " pH 4	140 hours no hydrolysis after 100 hours	7 hours no hydrolysis after 100 hours
LD 50 - mouse orally (mg/Kg)	200-250	27
I 50 - bovine cholinesterase	7.63×10^{-3} M.	3.91×10^{-5} M.
I 50 - fly-head cholinesterase	1.50×10^{-4} M.	1.71×10^{-7} M.

Effects of the Treatment on Insects and Mites

Citrus - Both dimethoate and the P=O derivative were applied at various dates and localities, on lemon, orange and tangerine trees, all heavily infested with scales in all stages. Both the leaves and the fruit were freed from Chrysomphalus dictyospermi, Mytilococcus Beckii and the fruit from Pseudococcus citri. Only a partial effect was obtained on Coccus oleae and a very slight effect on Aspidiotus haederae (Table 2). The application of the two products was completely effective on lemon plants badly infested with Tetranychus telarius (Table 3). However both products were practically useless against Aceria sheldoni on lemons, and exerted an inadequate control of adults of T. telarius which had been artificially transferred on leaves from orange trees previously treated (Table 3).

In Sicily where the two insecticides were applied to orange plants while the fruit was still green, we noticed that all the newborn larvae of Ceratitis capitata (which in Sicily die before penetrating the pulp, but which in other localities do irreparable damage to the pulp) died before leaving the egg-cavities in the outer portion of the peel (flavedo), while on the untreated plants the larvae penetrated into the inner colorless portion of the peel (albedo) (Table 4).

Olive - The treatment was effective on Dacus oleae larvae when the fruit was artificially infested in two different seasons (Table 5). An excellent control was obtained in November on leaf mining larvae of Prays oleellus (Table 5), while winter applications, when the larvae had already started to damage the leaves, were completely ineffective.

Apple - A rather inefficient control of Metatetranychus ulmi, and an incomplete effect on T. telarius were obtained with both products on leaves artificially infested with mites and taken from trunk treated plants in two experiments carried out on the same days in different localities (Table 6). A preblossom trunk application of dimethoate was completely ineffective against M. ulmi.

Table 2. Citrus. Toxicity of leaves and fruit to citrus scales and mealybugs after trunk applications of dimethoate and P=O derivative.

Plants	Active Ingredient (grams per tree)	Dates of applications and localities	Days after treatment	Average No. of Live Scales					
				Check		dimethoate		P=O derivative	
				per leaf	per fruit	per leaf	per fruit	per leaf	per fruit
Lemon[a]	10	Sept. 16	*Aspidiotus hederae* (adult females)						
		Catania	40	9.5	4.8	12.1	5.6	6.5	0.4
			80	44.0	48.4	20.1	4.0	16.1	0.0
Lemon[a]	20	Sept. 22	*Pseudococcus citri* (all stages)						
		Catania	4	-	30.5	-	15.5	-	21.5
			16	-	45.6	-	5.3	-	3.2
			32	-	32.3	-	0.4	-	0.1
Orange[a]	20	Aug. 17	*Chrysomphalus dictyospermi* (adult females)						
		Catania	32	38.2	-	0.9	-	0.3	-
			42	58.2	>1,800	0.2	0.0	0.1	0.0
			69	44.2	>1,600	0.0	0.0	0.0	0.0
			74	42.6	>2,000	0.0	0.0	0.0	0.0
Orange[b]	5	Sept. 8	*Mytilococcus Beckii* (adult females)						
		Orbetello	29	6.5	42.5	0.0	0.0	1.3	0.0
			51	6.1	76.0	0.0	0.0	1.5	0.0
Orange[b]	2.5	Oct. 7	*Coccus oleae* (2nd and 3rd instar)						
		Orbetello	22	22.9	78.8	0.9	0.1	0.7	0.0
Tangerine[a]	25	Sept. 15	*Pseudococcus citri* (all stages)						
		Catania	8	-	145	-	8.3	-	1.2
			18	-	176	-	0.2	-	0.0
			32	-	67	-	0.0	-	0.0
Tangerine[b]	2.5	Oct. 7	*Mytilococcus Beckii* (adult females)						
		Orbetello	22	15.2	24.4	0.1	0.1	0.0	0.0
			Coccus oleae (2nd and 3rd instar)						
			22	18.5	21.6	13.4	8.8	0.6	0.4

[a] - Average height : 3 meters
[b] - Average height : 1.5 meters

Table 3. Lemons and oranges. Toxicity of leaves and fruit to Tetranychus telarius after trunk applications of dimethoate[a] and P = O[a] derivative.

Plants	Dates (1959) of applications and localities	Days after treatment	Effect on Mites			
			dimethoate		P=O derivative	
			leaves	fruit	leaves	fruit
			Percent reduction of infested leaves on fruit			
Lemon[b]	Sept. 22 Catania	4	58	0	85	50
		16	99.5	100	100	100
		32	100	100	100	100
			Percent mortality of mites[c]			
Orange[b]	Aug. 17 Catania					
		4	98.5	-	99.0	-
		8	98.8	-	99.0	-
		16	99.8	-	96.5	-
		32	32.0	-	60.0	-

[a] - 20 grams per tree
[b] - Average height : 3 meters
[c] - On detached leaves infested with adult females of T. telarius

Table 4. Oranges. Toxicity of fruit to newly hatched larvae of Ceratitis capitata after trunk applications of dimethoate[a] and P = O derivative[a].

Treatment[b]	Percent Dead Larvae in the Ripe Fruit[c]		
	flavedo (in the egg cavities)	albedo	total
Dimethoate	100	0	100
P = O derivative	100	0	100
Check, no treatment	39.1	54.8	93.9

[a] - 8 grams per tree; average height : 2.5 meters
[b] - Date and locality of application : October 13, 1959, at Catania.
[c] - 32 days after treatment.

Table 5. Olives. Toxicity of fruit and leaves to largae of Dacus oleae and Prays oleellus respectively, after trunk or branch applications of dimethoate and P=O derivative.

Active Ingredient (grams)	Dates (1959) of applications and localities	Days after treatment	Percent Dead Larvae in the Fruit or in the Leaves	
			dimethoate	P=O derivative
			Dacus oleae[c] (in the fruit)	
10 (per branch of the same tree[a])	Jul. 27 Grosseto	44	99	87
		64	100	43
		85	51	5
			Dacus oleae[c] (in the fruit)	
20 (per tree[b])	Sept. 7 Firenze	11	76	95
		18	78	100
		23	81	100
		29	100	100
		37	89	98
		44	83	100
		66	77	92
			Prays oleellus (in the leaves)	
20 (per tree[b])	Nov. 21 Firenze	32	65	96
		76	99.5	100

[a] - Average height : 3.50 meters; "Ascolana" variety (eating-olives).
[b] - Average height : 3.50 meters; "Frantoio" variety (oil-olives).
[c] - On fruit samples picked and artificially infested with D. oleae.

Table 6. Apples. Toxicity of leaves to Tetranychus telarius and Metatetranychus ulmi after trunk applications of dimethoate and P=O derivative

Active Ingredient (grams per tree)	Dates (1959) of applications and localities	Days after treatment	Percent Dead Mites (adult females) on Detached Leaves	
			dimethoate	P=O derivative
			Tetranychus telarius	
30[a]	May 26 Bologna	8	48	80
		15	32	63
			Metatetranychus ulmi	
		8	70	83
		15	48	46
			Metatetranychus ulmi	
10[b]	May 27 Firenze	3	25	-
		5	30	-
		12	90	-
		36	10	-
			Tetranychus telarius	
		5	90	-
		12	100	-
		36	81	-

[a] - Average height of trees : 6 meters.
[b] - Average height of trees : 3 meters.

Pear - Dimethoate, applied on the trunk, inhibited the development of the C. capitata larvae in artificially infested fruit.

Cherry - The application of dimethoate when the fruit was still green (Table 7) resulted in a great reduction of cherries infested by Rhagoletis cerasi larvae. This control seemed better than that obtained with later application when the cherries were changing color and the infestation had already started (Table 7). On the other hand, we had negative results on leaves, from the same plants, infested with T. telarius.

Peach - Following repeated positive results from preliminary tests on C. capitata carried out by means of artificial infestations, we tried an experiment under natural conditions. When applied while the fruit was green (about 24 days before ripeness) dimethoate was able to inhibit almost completely the development of the larvae in the peaches (Table 8). The results

of this Table show also, for each plant, the effects of different dosages of insecticide, and the erratic performance of one of them. The results on Hyalopterus pruni (Table 9) were completely positive with both compounds though on the same leaves T. telarius survived. Dimethoate appeared quite ineffective against Quadraspidiotus perniciosus and Pseudaulacaspis pentagona.

Considering the results in toto (Table 10), we can arrive at the following conclusions:

For certain species of pest there is a considerable variation in the results depending on dosage and date of application. The most susceptible pests were certain species of scales (which feed on the sap from the leaves and from the epicarp of citrus fruit); aphids, (feeding on the sap from leaves of the apple, cherry and peach trees); dipterous larvae, (which develop in the mesocarp of peaches, cherries and olives); and microlepidopterous larvae, (which, like leaf miners, feed on the parenchyma of the leaves of the olive).

Table 7. Cherries. Toxicity of fruit to larvae of Rhagoletis cerasi after trunk applications of dimethoate.

Dimethoate (grams per tree[a])	Percent Infested Fruit	Percent Dead Larvae in the Ripe Fruit[b]				Percent Reduction of Infested Fruit[c]
		first instar	second instar	third instar	Total	
		Treatment: May 17, 1960 (green fruit)				
12	96.5	90.7	0.5	0	91.2	80.3
24	97.0	94.8	0.5	0	95.3	86.2
48	99.5	88.5	0.5	0	89.0	92.7
		Treatment: May 31, 1960 (fruit changing colour)				
12	97.0	48.5	1.0	1.0	50.5	70.6
48	98.5	33.5	21.3	5.1	59.9	77.8
		Check, no treatment				
-	99.0	1.0	0	0	1.0	-

a - Average height of trees (Durello variety) : 8 meters.
b - 200 cherries per tree examined.
c - 2000 cherries per tree examined.

Table 8. Peaches. Toxicity of fruit to larvae of Ceratitis capitata after trunk applications of dimethoate.

Trees[a] No.	Days after treatment[b]	Percent Infested Fruit	Percent Dead Larvae in the Fruit			
			first instar	second instar	third instar	Total
			dimethoate: 8 grams per tree			
1	6	50	33	0	0	33
	10	60	72	0	0	72
	17	100	100	0	0	100
	24	100	95.2	4.8	0	100
2	6	0	0	0	0	0
	10	70	39.3	0	0	39.3
	17	90	47.5	5.4	0	52.9
	24	100	85.7	3.8	6.6	96.1
			dimethoate: 20 grams per tree			
3	6	40	36	0	0	36
	10	80	64.7	0.8	0	65.5
	17	80	100	0	0	100
	24	100	91.4	8.6	0	100
4	6	40	25.4	0	0	25.4
	10	70	52.6	0.6	0	53.2
	17	100	86.0	14.0	0	100
	24	100	100	0	0	100
			check: no treatment			
5	6	30	0	0	0	0
	10	50	0	0	0	0
	17	100	0	2.8	0	2.8
	24	100	0	14.8	0	14.8

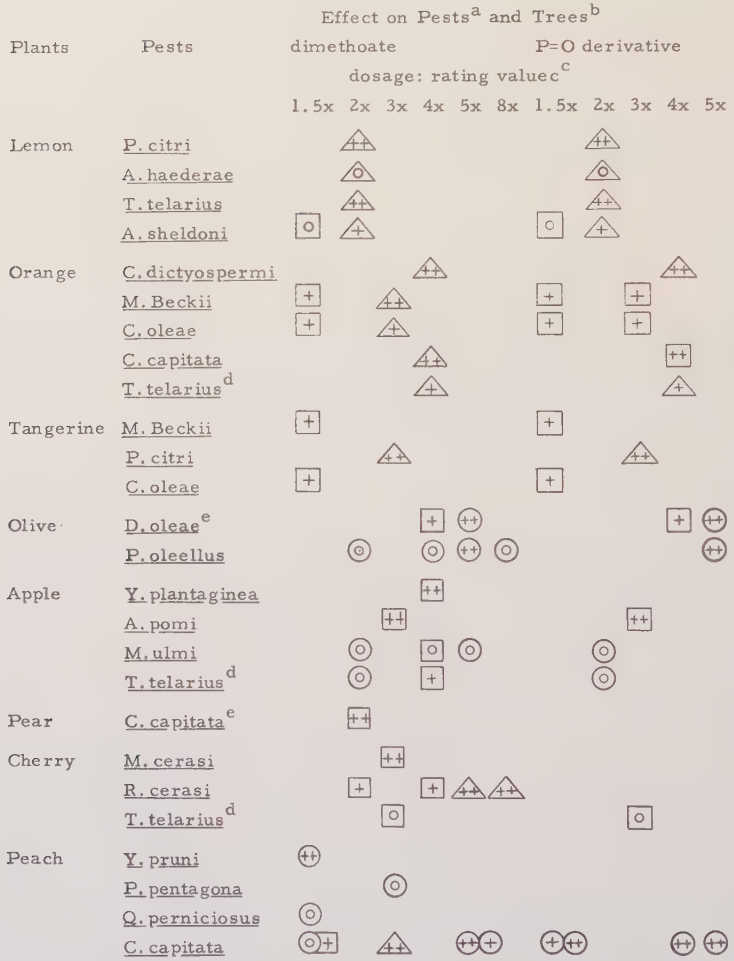
[a] - Average height of trees ("Hale" variety) : 3 meters.
[b] - Treatment : July 13, 1960 at Catania; fruit was completely green.

Table 9. Peaches. Toxicity of leaves to Hyalopterus pruni and Tetranychus telarius after trunk applications of dimethoate[a] and its P=O[a] derivative.

Days After Treatment[b]	Effect on Aphids and Mites	
	dimethoate	P=O derivative
	Hyalopterus pruni (percent reduction of infested leaves)	
1	0	10
3	34	94
4	90	96
6	100	100
13	100	100
29	100	100
60	100	100
	Tetranychus telarius (percent mortality of adult females)	
4	41	90
13	22	56
30	8	8

a - 5 grams per tree ("Alexander" variety); average height: 3 meters.
b - treatment : May 26, 1959, at Florence.
c - on detached leaves.

Table 10. Relation of effectiveness and phytotoxicity of dimethoate and its P=O derivative to several species of pests and trees and to dosage applied.



[a] - Effectiveness. Rating values: ++ complete control; + fairly good control; o = inadequate or no control.

[b] - Phytotoxicity. Rating values: △ severe injury; □ moderate injury; ○ = slight or no injury.

[c] - Dosage. Rating values: Times (1.5x ... 8x) that the dosage of active ingredient applied on the tree trunk is superior to the dosage which could be applied by full coverage spray.

[d] - on detached leaves. [e] - fruit artificially infested. [f] - Insecticides applied on the branches.

The most unsuccessful results, on the other hand, were to two species of scales (P. pentagona and Q. perniciosus) which feed on the cortical tissues of the branches and trunk, and three species of mites which feed on the content of first layers of cells below the epidermis of the leaves (M. ulmi and T. telarius) or of the buds (A. sheldoni).

The activity of the two insecticides does not differ substantially, but it was noted that the P=O derivative acted in many cases with greater rapidity than dimethoate.

Distribution of the Substances Translocated into the Plants

The concentration of the insecticide in leaves and fruit was assessed at various intervals of time, by means of bio-assays, on the same plants used for the insect and mite tests described above.

The results are shown, in graphic form, as follows: lemons (Fig. 1 and 2 above), tangerines (Fig. 2 below), oranges (Fig. 3), olives (Fig. 4), cherries and apples (Fig. 5). In order to facilitate comparison, the two variables (time and concentration) are plotted on semi-logarithmic paper: segments of lines having equal slope with respect to the abscissa, represent, therefore, equal relative variations. Despite the changes in the values and relative variations in the concentrations of insecticide in the leaves and in the fruit (depending on kind of plant, time of application and product applied), there are some uniform tendencies which can be summarized as follows:

The maximum concentration of insecticide which reaches the leaves is notably higher than that in the fruit (Figs. 1, 2, 3 below, 5). There was only one exception (Fig. 3 above).

In citrus plants, the insecticide accumulates much more in newly formed leaves than in old leaves (Figs. 2 above, 3 below).

For the whole or greater part of the experiment, maximum concentrations of insecticide are found in the peel of citrus fruit, which are higher than those found in the pulp (Figs. 1, 2, 3). Moreover in ripe fruit the concentration is less than that found in green fruit (Fig. 1).

Considering the variations with time, it seems that the concentration of insecticide having reached a maximum in leaves and fruit (more frequently first in the leaves and then in the fruit), diminishes in both. In the leaves this happens more rapidly in most cases than in the fruit (Figs. 1, 2, 3, 5). The curves which express the insecticide content in the vegetative tissue, at different times, are almost always asymetric: the maximum concentration occurs in a short period of time, while a variable but longer period is necessary to achieve the minimum values (less than 0.1 p.p.m.) which can be regarded as having no pesticidal and toxicological meaning.

Even though the behavior of the products is similar in a general way, several differences distinguish dimethoate from the P=O derivative (Figs. 1, 2, 3, 4). The difference between the concentrations of the insecticide in the leaves and in the fruit of the plants treated with the P=O derivative

is less than in those treated with dimethoate. Furthermore the maximum concentration of the insecticide is attained in a shorter time with the P=O derivative, and the decreases are more rapid. In particular the decrease in the peel of citrus fruit takes place faster than in the pulp, so that some time after the application of the P=O derivative, the concentration in the pulp is higher than in the peel.

More detailed aspects of the distribution of compounds translocated into the leaves and fruit are shown in an experiment made by applying P^{32}-labelled dimethoate to the trunk of a peach tree and determining: a) the concentration of radioactive phosphorus percent as dimethoate and its degradation products (total P^{32}) and b) the concentration of radioactive phosphorus which can be extracted by means of chloroform (Santi, in prep.) and present as dimethoate and its P=O metabolite ($P^{32}/CHCl_3$).

The graphs in Fig. 6 again show that the concentration of both total P^{32} and $P^{32}/CHCl_3$ reaches and maintains higher levels in the leaves (greater in the terminal leaves) than in the fruit. Moreover, while the total P^{32} content in leaves reaches a maximum and then very slowly decreases and, in peaches, the increase continues until ripeness, the concentration of $P^{32}/CHCl_3$ after having reached its maximum in the leaves and a little later in the fruit, decreases rapidly in both. Further, even though the $P^{32}/CHCl_3$ content of terminal peaches is not always greater than that of fruit picked near the base of twigs, the same substances (mainly dimethoate and the P=O metabolite) appear more concentrated in the half of the fruit near the stem. This pattern of distribution, which is due to the penetration of the radioactive compounds through the stem end, has been confirmed by autoradiograms of the section of peaches (Fig. 7), which differ substantially from that obtained from fruit sprayed with P^{32}-labelled dimethoate (Fig. 7). The autoradiograms of peach leaves (Fig. 8) show that the substances containing labelled phosphorus, which penetrate through the petiole and the midrib, distribute themselves initially in the tissues between the secondary veins and then localize progressively along the borders of the leaf blade.

Adverse Effects on Plants

The adverse effects following the application of the two insecticides dissolved in TBF are shown by symptoms which generally begin to appear many weeks or months after the date of treatment. They appear both at that part of the trunk where the product was applied (lesions in the outer layers of the bark, cortical necrosis, injuries to the conducting tissues) and in the foliage (slowing-down of growth, chlorosis, phylloptosis). The nature of the symptoms and the slowness with which they become apparent, in contrast to the rapidity of damage when excessive dosage of the same insecticides is used as a spray, leads to the hypothesis that the cause of adverse effects after trunk application can be traced to lesions on the treated part of the trunk with results, in the most severe cases, which can be compared to those caused by girdling.

A summary of the phytotoxic effect to various kinds of plants, many months and even almost two years after the date of treatment, is depicted in Table 10, in which are also shown the relative dosages of the product used and the corresponding insecticidal and acaricidal activity. Some uniformities are evident: within the limits of the dosages tested, it would seem that the adverse effects are more closely related to the botanic species than to the dosage of the product. In fact, although we noted a certain variance in results on the same species of plants (with regard to dosage and other factors not easily ascertainable), citrus trees were badly damaged even with relatively low dosage; cherry, pear and even more so apple trees reacted less to the same dosage; peach trees were even more tolerant with only two exceptions and, finally, olive trees were the only plants to tolerate dosages of the two insecticides which were heavy enough to control D. oleae and P. oleellus effectively.

Of the two products dimethoate appears to be more phytotoxic, especially to citrus, than does the P=O derivative.

Influence of the Formulation on the Effect on Pests and Plants

In reviewing the results of the experiments with dimethoate and its P=O derivative in TBF from the practical viewpoint, two positive aspects emerge: the effective control of several species of pests and, which is peculiar, the minimum residue of insecticide in the fruit. However, a negative aspect has to be mentioned: the absence of an adequate and constant margin of safety, in many of the plants studied, between the effective insecticidal dose and that causing damage.

Tests with P^{32}-labelled dimethoate in TBF, applied to the stem of potted sour orange seedlings, have shown that, by varying the concentration of active ingredient in the formulation and varying consequentially, the amount of trunk surface to be painted, neither the phytotoxicity nor the speed of translocation of the penetrated substances changes appreciably, provided that the same amount of active principle is used.

We continued with further experiments varying the type of solvent. Tests, carried out with potted apple trees infested with Aphis pomi (Fig. 9), demonstrated that the speed, with which both dimethoate and its P=O derivative control aphids, is a function of the solvent, the greater speed being attained with TBF. The same conclusion was reached with the application of dimethoate in different solvents on peaches infested with H. pruni (Fig. 9). But while on peaches none of the formulations tried gave adverse effects, on the apples treated with both products in TBF, the painted portion of the bark of the stem appeared severely injured.

There is evidence, therefore, of a correlation between the speed of appearance of insecticidal effects and phytotoxicity.

More extended tests were made on sour orange seedlings in pots which proved particularly susceptible to phytotoxic effects. The results showed that while none of the ten solvents tested could itself be blamed for the adverse effects, the addition of dimethoate (Table 11) caused injury, the severity of which was dependent on the solvent and the dosage of the active

ingredient. Heavy damage was caused by dimethoate in TBF at all dosages tested, while lower doses of the same product diluted with other solvents (cyclohexanone, methyl cellosolve, methanol, methyl phosphate) were practically innocuous. Further experiments, by means of radiometric and autoradiographic techniques, have established that even the least damaging among the quoted solvents, permits the transfer of P^{32}-labelled dimethoate and its metabolites from the trunk to the leaves.

We can, therefore, conclude that the seriousness of the effect is positively related to the speed of translocation of the active ingredient and to its maximum concentration in the leaves. This conclusion represents a starting point for further studies.

Table 11. Phytotoxicity of several formulation of dimethoate (30%) with different solvents (70%) applied on the stem of sour orange potted seedlings.

Solvents	Phytotoxicity[a] dosage applied (μg)					
	750		1500		3000	
Butyl phosphate	△	△	△	△	△	△
Ethyl phosphate	o	o	△	□	▯▯	△
Methyl phosphate	o	o	o	□	△	□
Propyl phosphate	o	△	△	▯▯	△	△
iso-Propyl phosphate	△	□	△	□	△	△
iso-Butyl phosphate	o	o	△	△	△	△
Methanol	o	o	o	o	□	△
Dioxane	o	▯▯	□	□	△	△
Methyl cellosolve	o	o	o	o	△	o
Cyclohexanone	o	o	o	o	△	△

[a] - Phytotoxicity. Rating values: △ = severe injury or dead plant; ▯▯ = heavy injury; □ = moderate injury; o = slight or no injury. Results were checked 5 months after treatment.

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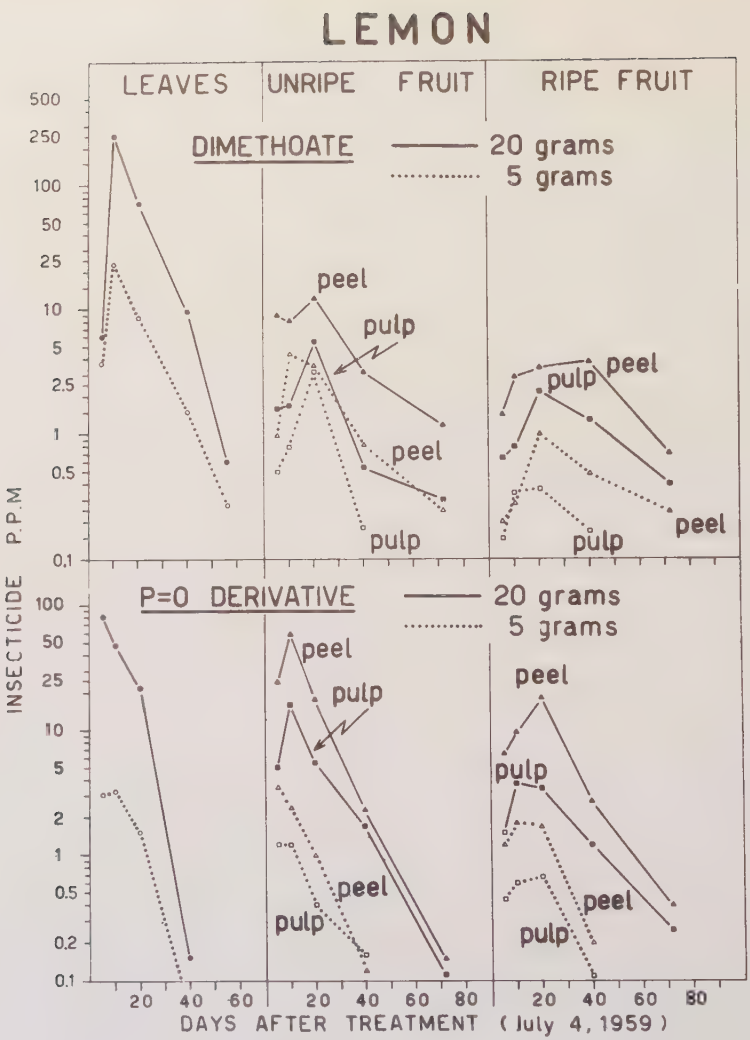

Fig. 1. Insecticide concentrations in the leaves and fruit of lemon trees after trunk applications of dimethoate and P=O derivative.

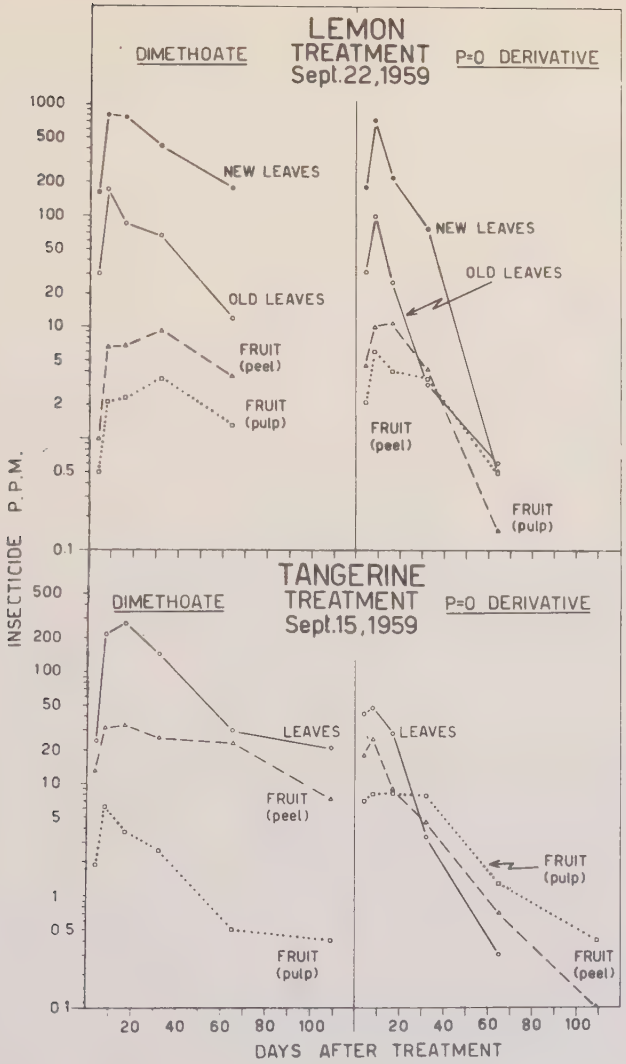

Fig. 2. Insecticide concentrations in the leaves and fruit of lemon (above) and tangerine (below) trees after trunk applications of dimethoate and P=O derivative.

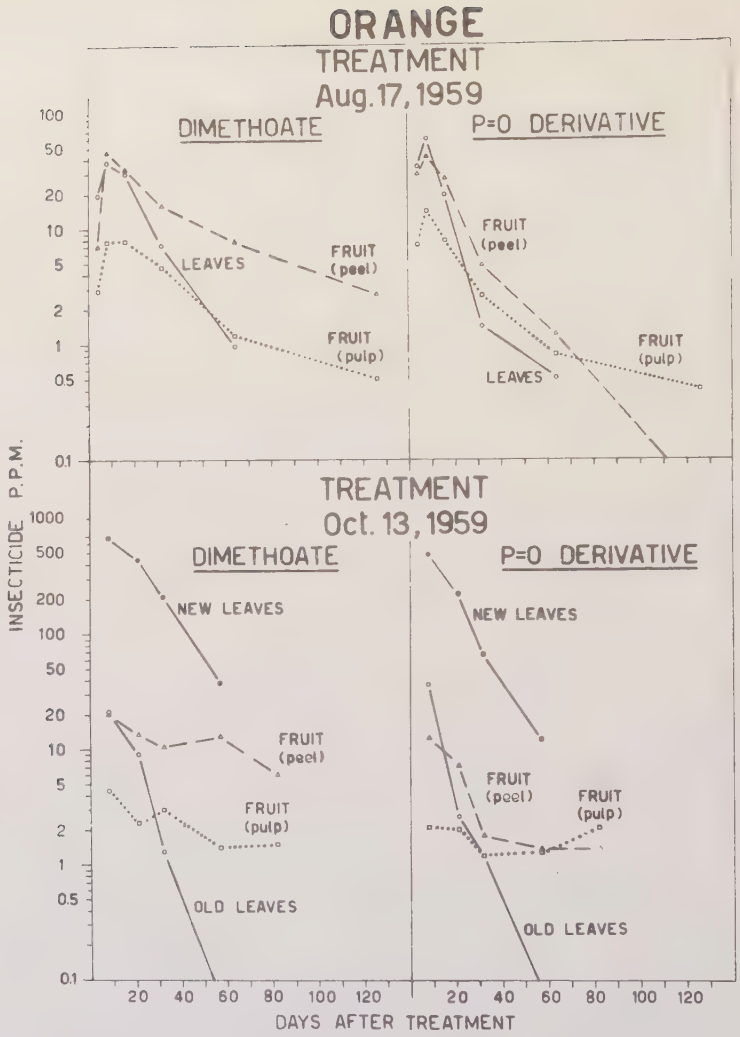

Fig. 3. Insecticide concentrations in the leaves and fruit of orange trees after trunk applications of dimethoate and its P=O derivative at two different dates.

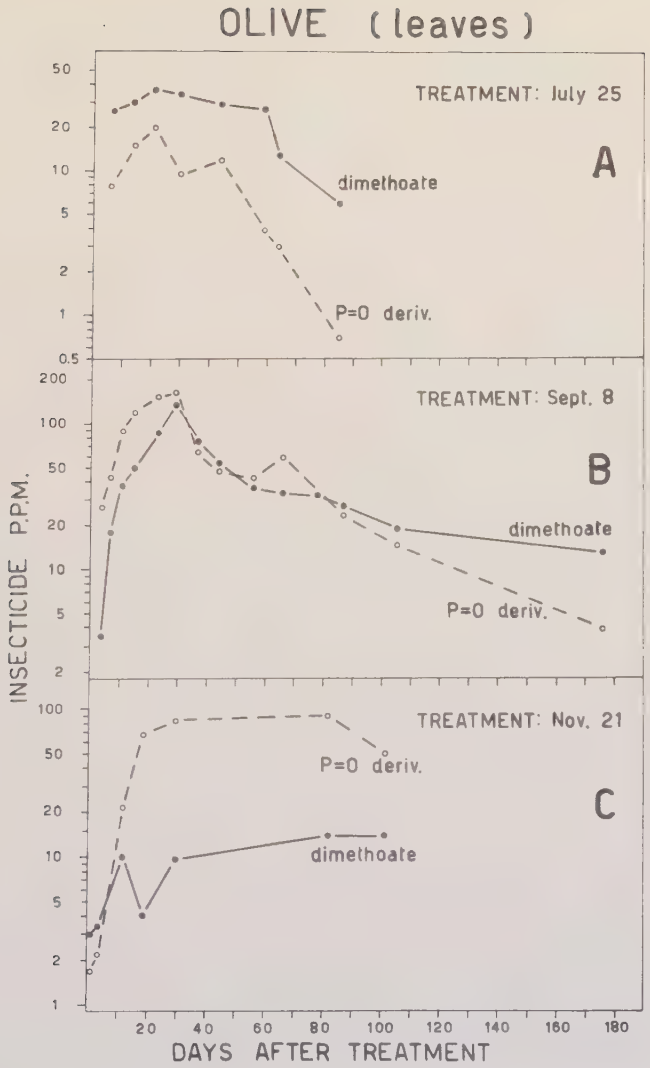

Fig. 4. Insecticide concentrations in the leaves of olive trees after branch (A) or trunk (B, C) applications of dimethoate, at three different dates.

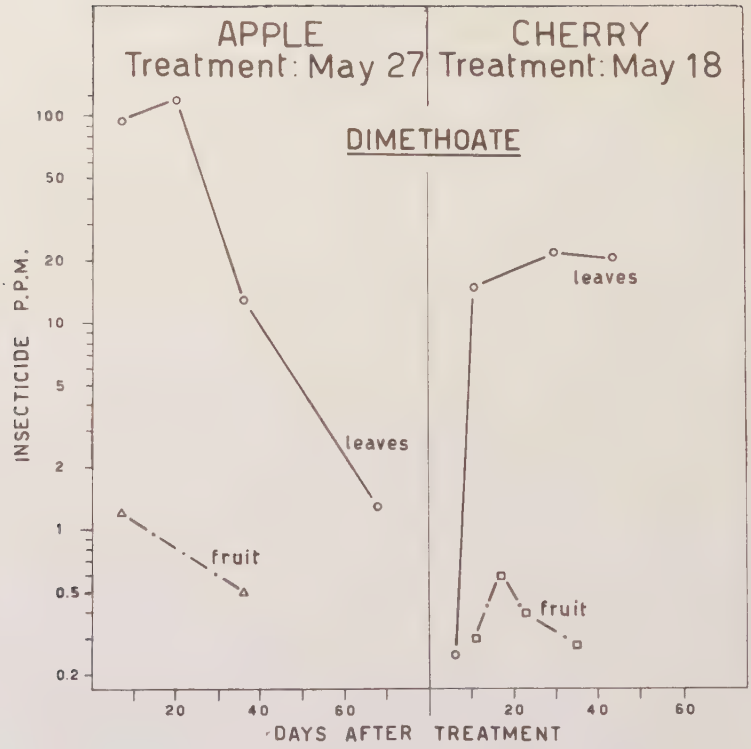

Fig. 5. Insecticide concentrations in the leaves and fruit of apple and cherry trees, after trunk applications of dimethoate.

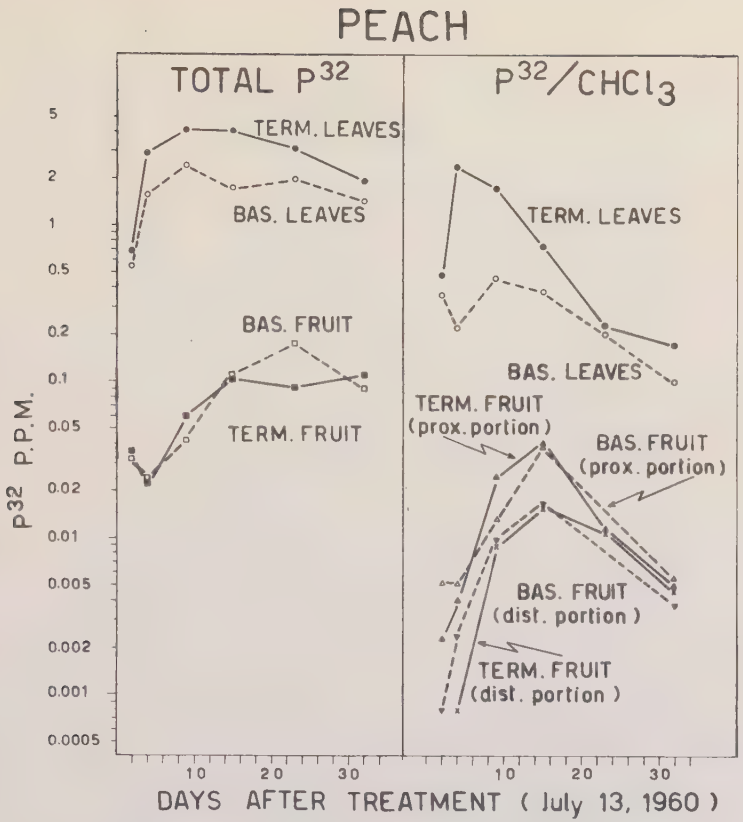

Fig. 6. Concentrations of total radioactive phosphorus (total P^{32}) and chloroform soluble radioactive phosphorus ($P^{32}/CHCl_3$) in the leaves and fruit of a peach tree after trunk application of P^{32}-labelled dimethoate.

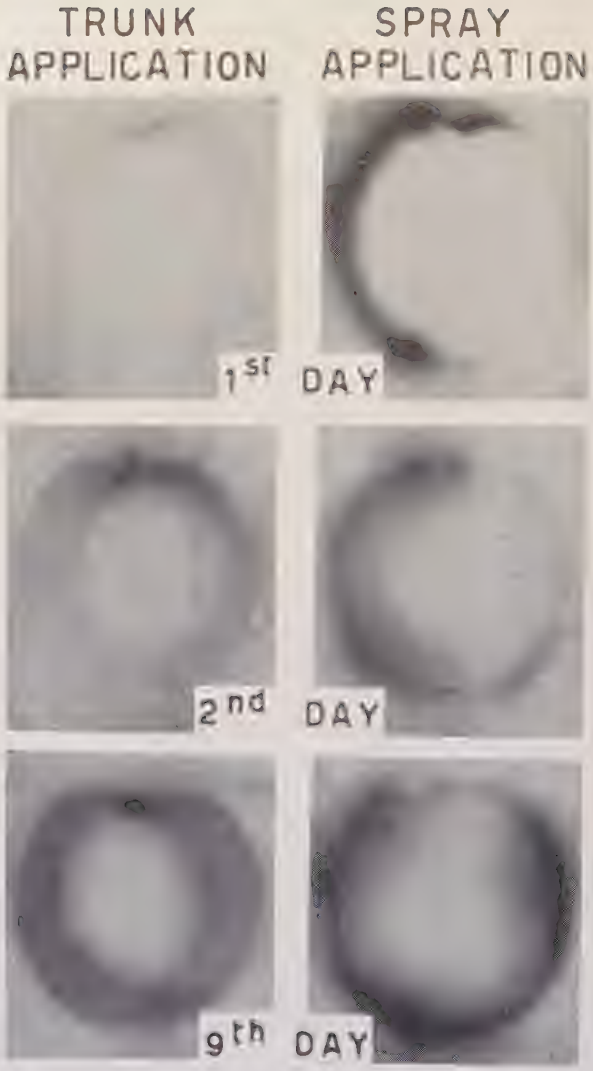

Fig. 7. Autoradiograms of longitudinal sections through peach fruits picked 1; 2, 9 days after trunk and spray applications of P^{32}-labelled dimethoate.

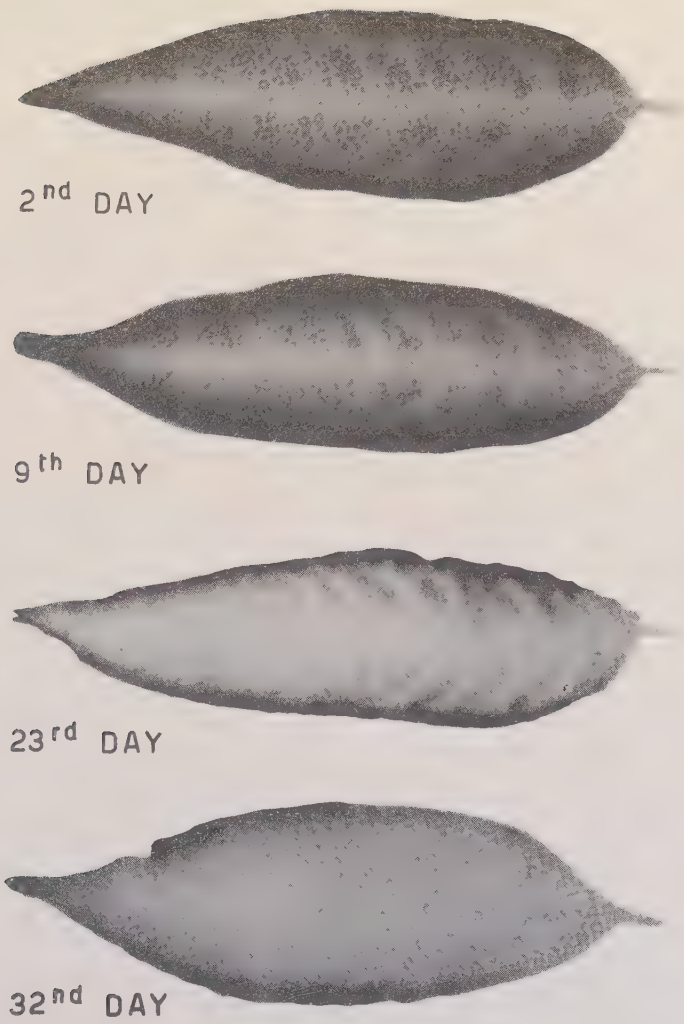

Fig. 8. Autoradiograms of peach leaves samples 2; 9; 23 and 32 days after trunk applications of P^{32}-labelled dimethoate.

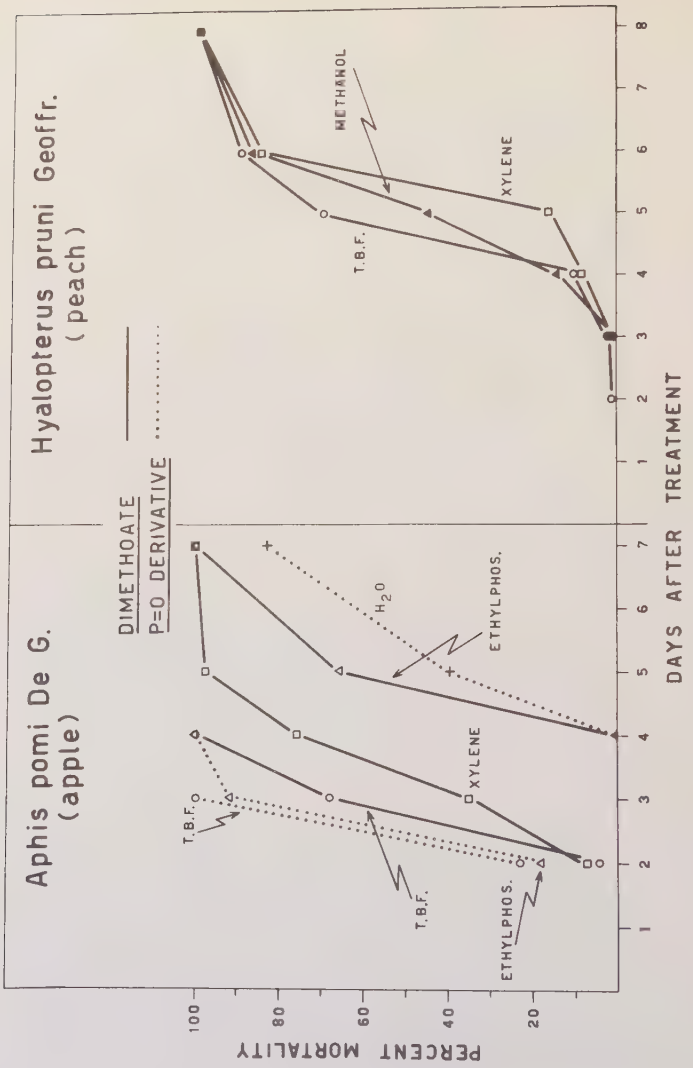

Fig. 9. Speed of action on Aphis pomi (apple tree) and Hyalopterus pruni (peach tree) of dimethoate and P=O derivative after trunk applications of the solution with different solvents: butylphosphate (T. B. F.), ethylphosphate, xylene, water, methanol.

MENAZON: DEVELOPMENT OF A SELECTIVE SYSTEMIC APHICIDE

by D. Price Jones
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Introduction

Recent trends in aphid control have been conditioned by two apparently conflicting requirements: 1, The need in some cases for a quick clean-up before harvest, with emphasis on a rapid reduction in residues of the aphicide and/or its metabolites; 2, The need in other cases for a more prolonged effect, with emphasis on the persistence of the aphicide for a few weeks with subsequent reduction of residues to safe levels.

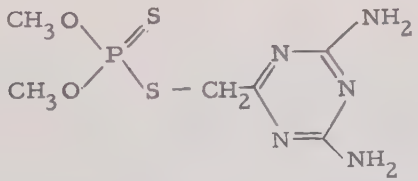
The former requirement applies for instance to the treatment of infested lettuce a few days before harvest and is not concerned with the prevention of damage. The latter requirement - persistence - is commercially the more important and applies to crops such as sugar beet and potatoes where the main objectives are the limitation of aphid populations and, often more important, the restriction of virus spread.

Menazon promises to meet the requirements of the second category. Its properties are discussed below and suggestions are made for the exploitation of these properties.

Chemical and Physical Properties of Menazon

The following account is intended to provide a suitable basis for the discussion of the biological properties. More information on chemical aspects may be found in a preliminary report by Calderbank, Edgar and Silk (1961).

Menazon is the BSI accepted common name for S-(4,6 diamino - 1, 3, 5 triazin-2-yl) methyl OO-dimethyl phosphorothiolothionate:



It is a white crystalline material with a melting point of 160° C. It has an extremely low - as yet undertermined - vapour pressure and, as a consequence, no fumigant properties. The technical material, in bulk, has a mercaptan like odour which is not apparent in aqueous spray dilutions.

Menazon is soluble in water to about 0.024 g/100 ml at 20 - 25° C. At 4 oz a.i./acre, it should be largely soluble in 100 gal water/acre, given a

sufficient interval between mixing and spraying. At lower volume rates, e,g, 3 gal/acre as in aerial spraying, very little of the menazon would be present in solution. Menazon is moderately soluble in hydroxylic solvents and only very slightly in other organic solvents.

The crystalline solid is stable at temperatures up to 50° C. The formulated material is stable on leaf surfaces and is also sufficiently stable in most soils to warrant its use as a soil or seed treatment. It is compatible with most commonly used insecticides and fungicides but should not be mixed with strongly alkaline materials.

Biological Properties

Mammalian Toxicity

As the development of a new material is inevitably governed by its mammalian toxicity, it is pertinent to compare menazon with other aphicides in current use (Table 1). In this comparison on rats, the only one readily available, menazon is shown to be one of the least toxic.

Table 1. Acute oral toxicity of important aphicides

Aphicide	Acute oral LD 50 mg/kg, rat
Menazon	1950
Malathion	1400-5800
Dimethoate	600
Lindane	125
Demeton-methyl	85-120
Nicotine	50-60
Parathion	6-15
Demeton	9
Mevinphos	7
Phorate	4
TEPP	2

Sources of toxicity data: Menazon: Imperial Chemical Industries; Dimethoate: Fison's Pest Control; Demeton-methyl: Baywood Chemicals; for other materials see Gunther and Jeppson (1961).

Investigations into other toxicological aspects, although not yet complete, are sufficiently advanced to establish criteria for commercial usage. Information has been submitted to the Advisory Committee on Poisonous Substances used in Agriculture and Food Storage and a clearance has been obtained for the use of menazon in 1962 on a number of crops, including sugar beet, potatoes, brussels sprouts, beans, hops, apples and strawberries, with a minimum interval of three weeks.

Mode of Action

Menazon is capable of killing aphids either by contact or by systemic action, the latter being in general the more important. Detailed physiological and biochemical investigations have yet to be undertaken. Present information is that menazon is a weak inhibitor of locust cholinesterase.

Spectrum of Activity

Menazon can fairly be described as a selective aphicide. While this selectivity is enhanced by systemic activity, it is in fact an inherent feature of the material as shown in direct contact toxicity tests in the absence of the host plant. It is important however to note that this selectivity is shown only at normal field dosage rates; at rates in excess of these, some kill of other insects may be achieved. The results of laboratory tests using menazon at different concentrations are given in Table 2. At the normal high volume concentration used on field crops, about 0.025%, only aphids suffered a total kill. However, Tetranychus, Dysdercus and Musca larvae were affected to some extent. At the highest concentration tested Phaedon and Musca adults were also affected.

These laboratory indications of selectivity are confirmed by observations in the field. Most aphids on field crops are amenable to control at spray concentrations of about 0.025% menazon at 100 gal/acre, or at equivalent low volume rates. Certain species of mites are also susceptible at this dosage level. Otherwise all the indications are that the vast majority of insects are relatively resistant to menazon.

Since the selectivity of menazon is not absolute, some kill of beneficial insects may be expected under some circumstances. This has been demonstrated by Way (1960, 1961) and has since been confirmed at Jealott's Hill for syrphids. Results of toxicity tests on bees are given in Table 3. Regarded pessimistically, these results show that a significant mortality among syrphid larvae and bees may be expected under conditions of commercial usage. Examined more critically, they reveal considerable progress in the development of a selective aphicide. Whether reformulation can increase the selectivity still further remains to be seen. It is essential in any case to adopt a method of using menazon which will fully exploit the degree of selectivity inherent in the material.

The significance of broad spectrum insecticides in the production of 'flare-ups' and also in inducing insecticide resistance in aphids in closed ecological communities has been discussed by Stern and Reynolds (1958). The integration of chemical control with biological control of the alfalfa aphid has been admirably appraised by Stern, Smith, van den Bosch and Hagen (1959).

Systemic Properties and Persistence

Menazon can be absorbed either through the roots or through the leaves and can move systemically to the upper parts of plants. Absorption into the leaf is relatively slow and is apparently influenced by the nature of the leaf surface, being apparently slower on waxy leaves. Penetration

Table 2. Menazon selectivity related to spray concentration

Pest Type of test	% Kill at concentration (ai) indicated				
	0.3%	0.1%	0.05%	0.025%	0.005%
Aphis fabae DC, RC, Sy	100	100	100	100	100
Acyrthosiphon pisi RC, Sy	100	100	100	100	100
Tetranychus telarius					
Walking stages DC, RC, St, Sy	-	100	-	95	20
Eggs DC	0	0	0	0	0
Dysdercus fasciatus RC, Sy	92	25	35	35	13
Phaedon cochleareae RC, St	40	0	5	0	0
Sitophilus granarius RC, St	0	0	0	0	0
Plutella maculipennis					
Caterpillars DC, RC, St	0	0	0	0	0
Musca domestica					
Adults DC, RC, St	30	30	0	0	0
Larvae DC, RC, St	100	70	-	25	-

Type of test: DC - direct contact, RC - residual contact,
St - stomach, Sy - Systemic

into the leaf may also be influenced by rainfall. Absorption into the plant by this route is very clearly dependent upon the amount of foliage present. Thus control of oat apple aphid is poor at bud burst, much better at green cluster and excellent in late summer. A similar progression has been noted with Myzus persicae on peach. Absorption through the roots, as with other systemics, leads to a more effective distribution within the aerial part of the plant, particularly into the new growth.

Little is known as yet about the mechanics of translocation and redistribution, although some interesting suggestions are beginning to emerge. For example, it is now becoming apparent that menazon deposited on the leaf surface forms a local reservoir from which active material can move into the leaf. The chemical stability of menazon and the resistance to weathering conferred by certain formulations, coupled with relatively slow absorption, can lead to a marked persistence of effect. A most striking example is given by the ability of a spray applied to apples on August 19th to give control of return migrants of the oat apple aphid even as late as 24th October. It is in fact, becoming apparent that the persistence of the aphicidal action of menazon is dependent upon the creation of such a reservoir on the leaf surface or in the soil. The half-life in the plant, judging by the results of recent biochemical work on young, actively growing tomatoes and beans, is only about 7 days. An external reservoir is therefore essential.

The redistribution of menazon is believed to be somewhat slower than that of certain other commonly used aphicides. Its performance is therefore likely to be relatively better against those aphids which show a preference for the older leaves. It does not appear to be capable of downward translocation in aphicidally significant amounts. It can however move from leaf to stem and thence to higher leaves on, for instance, broad bean plants. In comparison with demeton-methyl, such redistribution is slower but more persistent. There is a possibility that some redistribution on brussels sprouts is achieved by the movement of water from the leaves down the grooved petioles to the sprouts.

Phytotoxicity

Menazon has been tested in the field on a wide range of plants under varied climatic conditions. The general picture which has emerged so far is one of very low phytotoxicity. In particular, as a foliage spray it has proved safe at all normal dosage rates (4 - 8 oz per acre) on all the main crops, about thirty in all, including potatoes, sugar beet, beans, brassicas, cereals, apples, blackcurrants and peaches, and on a variety of ornamental plants, including roses.

As a seed dressing, it is harmless to most seeds even at relatively high dosage rates. For example no phytotoxicity has been noted in sufar beet at 2 and 4% W/W menazon on the seed, field beans and broad beans at 0.2 and 0.4%, alfalfa at 4%, cauliflower at 4%, cotton at 4%. On barley in one laboratory trial very slight tip scorch was produced presumably by the high concentration of menazon in the guttation droplets. On lettuce a

Table 3. Toxicity of systemic aphicides to honey bees

Aphicide	% Kill after 48 hours	
	Stomach poison (1)	Contact sprayed foliage (2)
Menazon	0	53
Demeton-methyl	10	77
Dimethoate	100	100
Control	0	0

(1) Bees fed on sugar syrup containing 5 p.p.m. a.i.
(2) Bees confined on dry foliage freshly sprayed at about 6 oz a.i./acre.

slight delay in emergence has been noted at 4% but in carrots phytotoxicity has occurred at 2%. On potatoes some slight delay in emergence has been noted in three trials but it is not known yet to what extent this is due to phytotoxicity or to mechanical damage to sprouts during the extra handling of the seed during treatment.

Granules applied to the soil at $\frac{1}{2}$ - 2 lb menazon/acre were not injurious to hops as a hill treatment, sugar beet and beans as a drill treatment or potatoes as a furrow treatment.

Drenches have been applied to the soil around hop hills at 4 lb menazon/acre, to beans in pots at 2 lb/acre, tobacco at transplanting at $\frac{1}{2}$ and 1 lb/acre and roses at indefinite but high rates, in all cases without indications of phytotoxicity.

Odour and Low Taint Potential

The technical material is somewhat malodorous in bulk but is relatively inoffensive when formulated and the spray fluid at normal concentrations is quite innocuous.

Tasting tests have been conducted with fruit and vegetables, including apples, peaches, strawberries, potatoes, brussels sprouts, lettuces and beans, so far without a hint of flavour changes. The short life of menazon within the plant and the even shorter life of its main metabolite suggest that the taint potential is very low. Nevertheless, tasting tests will continue for some time to come.

Development of Menazon

The development of menazon has already been in progress for about three years and a considerable amount of information has now been ac-

quired about the control of specific aphids. Nevertheless with new formulations, further information on biochemical aspects and the emergence of new political attitudes towards the use of chemicals in the field, the true position of menazon is only now becoming clear. This position is reviewed below with only brief reference to the results of recent trials. Full results will be reported elsewhere.

Seed Dressings

In laboratory tests, seed treatment with menazon has given protection against aphids for varying periods to alfalfa, barley, beans, carrots, cauliflowers, cotton, lettuce and sugar beet.

In the field good protection of beans against Aphis fabae for two months after spring plantings has been obtained with dosage rates of 0.2 and 0.4% menazon W/W. Likewise delay in the spread of viruses, mainly pea leaf roll has been obtained in beans, presumably through the control of pea aphid.

In sugar beet, Bonnemaison (1960) has reported up to 22 days protection against Myzus persicae with dosages of 0.8 - 1.6% W/W. In the U.K. in one trial, virus yellows incidence in August was reduced to half that in the control by seed treatment at 2% menazon W/W.

Foliage Sprays

Encouraging results have been obtained against aphids on an extensive series of crops, using in the majority of cases a dosage rate of about 4 oz menazon/acre on field crops and 8 - 12 oz on top fruit. The following are the more important crops and aphids, on which good, and in some cases excellent, results have been obtained: potatoes: Various leaf aphids; beans: Aphis fabae; sugar beet: Aphis fabae and Myzus persicae, with consequent reduction in yellow virus; brussels sprouts: Brevicoryne brassicae; groundnuts: Aphis craccivora; cotton: Aphis gossypii; sorghum: Longuinguis sacchari; tobacco: Myzus persicae; apple: Aphis pomi, Eriosoma lanigerum; cherry: Myzus cerasi; peach: Myzus persicae; citrus: Toxoptera aurentii.

In a different category are a few crops, with their attendant aphids, where results as yet have not been satisfactory. On hops for instance, menazon sprays have not produced a sufficiently quick initial kill although their persistence has resulted in the depression of aphid populations for long periods, compared with demeton-methyl. On strawberries aphid control has been rather poor. It has been suggested that the outer leaves shield the inner parts of the plants and that very little translocation to the centre occurs with any of the systemic compounds. However some of them, e.g. demeton-methyl, are apparently sufficiently volatile to have a fumigant effect not obtainable with menazon. As already mentioned, control of oat apple aphid at bud burst does not seem to be readily achievable, because of the limited absorptive surface.

Despite the slow initial action of menazon, excellent control of virus has been obtained on sugar beet. Nevertheless the slow initial action does

underline the need for early application of menazon in certain cases, e.g. on brussels sprouts.

The further development of menazon in foliage applications is likely to be conditioned to a large extent by its selectivity of action. On the one hand its use on crops such as apples and cotton will be restricted by the occurrence of pest complexes. On the other hand, its use on large areas of crops such as sugar beet, potatoes, beans and alfalfa will be favoured by its minimal toxicity to beneficial insects.

Acknowledgements

Acknowledgements are due to the many colleagues who have participated in this work and particularly to Mr. E. C. Edgar and Dr. A. Calderbank who have been responsible for most of the biological and biochemical work, respectively.

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MENAZON: CONTROL OF POTATO APHIDS AND APHID-TRANSMITTED VIRUSES BY TUBER TREATMENT

by J. F. Newman
Jealott's Hill Research Station

Introduction

In the last thirty years British potato stocks have greatly improved in their freedom from virus diseases. This has been achieved by certification regulations for seed produced on higher ground in the more northerly parts of the country. At the present time, about two-thirds of the ware potato acreage is planted with one of the grades of certified seed and most of the remaining area is planted with once grown seed. Cost of certified seed represents about one quarter of the total production cost of main crop potatoes, and about one third of the cost of growing earlies. Over the last ten years, experiments have been done, notably by Broadbent and others at Rothamsted Experimental Station, on the use of insecticidal foliage sprays to control the aphid vector of the important virus diseases and so to facilitate the production of virus free seed, and even to extend seed production into areas which would otherwise be unsatisfactory. This work has shown that a programme of foliage sprays with an efficient insecticide, starting at the time of 80% emergence of the sprouts and continued at fortnightly intervals into July can largely stop the spread of leaf roll and check the spread of virus Y. (Broadbent et al. 1956, 1960).

In 1960, menazon (Calderbank, Edgar and Silk, 1961) became available for field trial work. This compound shows high systemic aphicidal activity of long persistence, together with a low mammalian toxicity (approx. 2000 mg/kg to rats) and low phytotoxicity. Pot tests done early in 1960 having given favourable indication, field experiments were started to investigate the use of menazon treatments against potato aphids, and in particular to find the extent to which potatoes could be protected against virus disease spread by a single application of menazon used as a tuber dressing.

Experimental

Replicate plot trials

In 1960 replicate plot trials were done at Jealott's Hill, Berkshire and at Fernhurst, Sussex. In 1961, in addition to further trials at Jealott's Hill and Fernhurst, a similar trial was laid down at Rippingale Fen, Lincolnshire. In each of these trials five treatments were used in a Latin Square plan, with square plots, 1/40 acre in area, of side lengths equivalent to 16 rows of potatoes. The plots were separated on all sides by untreated guard areas of width equivalent to 9 rows. Twelve virus infected tubers, 6 leaf roll and 6 virus Y, were planted at regularly spaced intervals in each plot, giving approximately 1.7% of each virus.

Tuber treatments were done by spraying with dispersions containing, for the 1 lb/ton treatment, 2% or 4% of menazon at rates of 2 pints or 1

pint per cwt of seed respectively. The menazon content of the dispersion was adjusted appropriately for the other treatments. Where chitted seed was used, the application was made to the tubers in the normal chitting trays, loaded at 3 trays/cwt. Bagged seed was spread on the floor, sprayed in the same way and allowed to dry before being rebagged. Tubers were generally treated shortly before planting.

Granular treatments, using granules containing 0.5% of menazon on an inert base, were applied in the open furrows at the time of planting. Tins were cut to size to hold sufficient granules to treat 100 ft. row and the granules were shaken by hand from these tins into the rows.

Foliage spray treatments were applied by an eight nozzle hand boom sprayer, held by two persons, and operated through a pressure regulating valve from a pressurised knapsack sprayer. In all cases, the spray liquid was applied at 40 gal/acre, using Allman No. 00 jets at 20 lb/in^2.

Observations on aphid infestation were made by the method of Hollings (1955). A top, a middle and a lower leaf were examined for the presence of apterae on each of 20 plants per plot. Any plant on which an aphid was found was counted as infested. In some cases, counts were also made of the total number of apterous aphids on the sampled leaves, and these figures are shown bracketed in the tables.

At harvest time, samples of 300 tubers from each plot were taken and stored for growing on in the following year for assessment of virus disease. Observations on plants from the 1960 trials tuber samples were made in July 1961, and similar observations on the 1961 samples will be made in 1962.

Strip Trials

In 1961, in addition to the replicate plot trials, strip trials of greater area were done at several sites in Lincolnshire, Pembrokeshire, and Berkshire. In these trials, no attempt was made to assess virus disease spread, but aphid population observations were made as in the replicate plot trials. In one trial, at Grazeley Green, Berkshire, a mechanical planter was used, and a granule treatment was applied through the fertilizer section of the machine, the fertilizer having been applied previously broadcast.

Results

Results are shown in detail in the tables. Figures for aphid population in all cases refer to total potato aphids. Where the date of an observation is the same as a spray application date, in all cases the count was made before the spray was applied. In the trials in Pembrokeshire, no aphid attack occurred and no results were obtained.

Tuber Treatments

Tuber treatments were used at 1 lb/ton in 1960 and at $\frac{1}{2}$, 1 and 2 lb/ton in 1961. All treatments resulted in substantial aphid control extending into July in the case of main crop trials or until the potatoes were lifted in early crops. In the replicate plot trials where the comparison is available the degree of control maintained through the season seems similar to that produced by three foliage spray applications of demeton methyl or of menazon. In the strip trials at Threekingham and Folkingham the tuber treatments applied in the preceding autumn exerted considerable control until the crops were harvested on 23rd June and 7th July respectively.

The figures for the replicate plot trials at Jealott's Hill and Fernhurst, where early aphid attacks occurred, provide an interesting comparison between the tuber treatments and spray treatments in their ability to control an early attack. The first spray treatment in each trial was applied at the stage of approximately 80% emergence of the sprouts, at which time many aphids were present on the controls and on the plants to be sprayed, while on the tuber treatments the aphids were already well controlled. The control of early attack is most important in the limitation of virus spread and it is evident that the protection provided by tuber treatments right from the time the sprouts emerge from the soil is a considerable advantage arising from this application method.

Granule Treatments

Granule applications were made in 1961 in two replicate plot trials (Jealott's Hill, Fernhurst) and in two strip trials (Threekingham and Grazeley Green). In all these trials, the ultimate residual aphicidal action was similar to that produced by tuber dressings at comparable rates, but the granule treatments were somewhat inferior in the early protection of the emerging sprouts. The granules were scattered more or less evenly along the rows at planting, and hence the acquisition by the plant of a protective concentration of menazon in the sprout is presumably dependent upon sufficient root development to reach enough granules. The dry weather conditions in the early summer of 1961 may also have had some effect in limiting the release of menazon from the granules in the soil. It is clear that further experiments in the use of granules should aim to investigate the use of placement treatments in which the granules are grouped more closely around the seed tuber.

Spray Treatments

In the replicate plot trials, foliage spray treatments with demeton methyl, demeton S methyl and menazon were included mainly as standards for comparison with the soil systemic treatments. The menazon spray treatments at 4 oz/acre appeared to produce aphid control similar to that given by other established spray treatments.

Control of virus disease spread

Samples of tubers from the 1960 Jealott's Hill trial proved to be heav-

ily infected with leaf roll and virus Y when grown on in 1961. It is clear that the intensity of attack by virus infected aphids completely swamped any differences in spread within the crop.

Results for the Fernhurst 1960 trial are shown in Tables 9, 10 and 11. Infection in these samples was not heavy, but no significant differences occur between any of the treatments and the control. This result indicates that the infection arose from viruliferous winged aphids migrating into the crop from outside, a process which no insecticidal treatment applied to the crop can affect, and that little spread took place within the crop even in the absence of any insecticide treatment. A marked positional effect is noticeable in the incidence of leaf roll in this trial (Table 9), indicating arrival of infected aphids from a south-westerly direction. It should be noted that the test conditions used were severe, with minimal plot sizes in a small total trial area, and the gradient of infection indicates that more satisfactory virus control might be expected in large potato fields, particularly when isolated from untreated potato crops carrying virus infection.

These results, however, illustrate the fact that insecticide treatments alone are not likely to enable good quality seed potatoes to be produced for many years without regard to location and hence are not likely greatly to threaten the carefully established seed potato industries of Scotland and Northern Ireland and elsewhere. Insecticide treatments will rather, in due course, be of value as an adjunct to ecological and cultural practices in seed production.

Residues

Samples of ware tubers were taken at harvest from the 1960 replicate plot trials and subjected to chemical analysis for residues of menazon, using a method capable of determining 0.05 p.p.m. of menazon. No residues were detectable in any samples. Analyses were also done on tubers from plants sprayed with menazon at 8 oz/acre at either 8 weeks or $3\frac{1}{2}$ weeks before harvest. No residues were detectable.

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Table 1. JEALOTT'S HILL - Replicate Plot Trial - 1960
Planted 13th April 1960 : Variety - Majestic

Treatments (All rates refer to active ingredients)	Aphid observations at various dates			
	2/6	20/6	6/7	14/7
Tuber treatment - Menazon 1 lb/ton + foliage spray - Menazon 6 oz/acre on 7/6	11	24	73 (309)	25 (67)
Menazon foliage spray - 4 oz/acre x 2 applications on 7/6 and 15/6	35	13	64 (204)	34 (72)
Menazon foliage spray - 4 oz/acre x 3 applications on 26/5, 7/6 and 15/6	37	22	61 (207)	29 (52)
Demeton methyl foliage spray - 6 oz/acre x 3 applications on 26/5, 7/6 and 15/6	32	17	80 (306)	4 (5)
Control	83	92	100 (2482)	92 (739)

Note: Aphid observation - First figure gives % infested plants
Bracketed figure gives number of aphids per 300 leaves

Table 2. FERNHURST - Replicate Plot Trial - 1960
Planted 28th April 1960 : Variety - Majestic

Treatments (All rates refer to active ingredients)	Aphid observations at various dates			
	21/6	30/6	12/7	19/7
Tuber treatment - Menazon 1 lb/ton + foliage spray - Menazon 4 oz/acre on 12/7	9	34 (108)	35 (66)	4 (4)
Menazon foliage spray - 4 oz/acre x 2 applications on 1/6 and 22/6	76	46 (101)	48 (109)	20 (27)
Menazon foliage spray - 4 oz/acre x 3 applications on 1/6, 22/6 and 12/7	76	39 (87)	54 (115)	7 (11)
Demeton methyl - 6 oz/acre x 3 applications on 1/6, 22/6 and 12/7	69	28 (51)	51 (93)	1 (1)
Control	79	96 (739)	82 (409)	44 (121)

Note: Aphid observation - First figure gives % infested plants
Bracketed figure gives number of aphids per 300 leaves

Table 3. JEALOTT'S HILL - Replicate Plot Trial - 1961
Planted 21st April 1961 : Variety Majestic

Treatments (All rates refer to active ingredients)	Aphid observations at various dates						
	30/5	2/6	14/6	16/6	30/6	4/7	14/7
Tuber treatment - Menazon ½ lb/ton + 1 spray at 4 oz/acre on 30/6	12 (36)	16 (55)	52 (144)	63 (152)	73 (246)	24 (41)	12 (12)
Tuber treatment - Menazon 2 lb/ton	2 (7)	7 (12)	37 (58)	27 (63)	55 (148)	33 (61)	12.5 (15)
Granule treatment - Menazon granules in rows at planting at 2 lb/acre	25 (88)	18 (87)	34 (74)	31 (66)	66 (196)	47 (87)	10 (22)
Demeton S methyl foliage spray 3 oz/acre x 3 applications on 30/5, 14/6 and 30/6	72 (411)	4 (4)	67 (189)	6 (6)	58 (142)	3 (3)	7 (7)
Control	70 (343)	79 (405)	88 (464)	89 (651)	95 (735)	76 (287)	17 (17)

Note: Aphid observations - First figure gives % infested plants
Bracketed figure gives number of aphids per 300 leaves

Table 4. FERNHURST - Replicate Plot Trial - 1961
Planted 18th April 1961 - Variety - Majestic

Treatments (All rates refer to active ingredients)	Aphid observations at various dates				
	6/6	19/6	27/6	5/7	25/7
Tuber treatment - Menazon 2 lb/ton	21 (30)	41 (61)	38 (76)	31 (42)	10 (10)
Tuber treatment - Menazon 1 lb/ton	28 (30)	48 (82)	42 (83)	40 (64)	0 (0)
Menazon foliage spray - 4 oz/acre x 3 applications on 6/6, 19/6 and 5/7	57 (140)	46 (80)	17 (30)	19 (27)	8 (8)
Granule treatment - Menazon granules in rows at planting at 2 lb/acre	39 (85)	45 (82)	42 (115)	48 (95)	13 (13)
Control	59 (116)	51 (100)	72 (204)	70 (223)	0 (0)

Note: Aphid observation - First figure gives % infested plants
Bracketed figure gives number of aphids per 300 leaves

Table 5. RIPPINGALE FEN - Replicate Plot Trial - 1961
Planted 28th May 1961 :
Variety - Record.

Treatments (All rates refer to active ingredients)	Aphid observations at various dates			
	6/6	22/6	11/7	24/7
Tuber treatment - Menazon ½ lb/ton	1 (1)	41 (122)	18 (24)	0 (0)
Tuber treatment - Menazon 1 lb/ton	0 (0)	34 (72)	16 (28)	0 (0)
Tuber treatment - Menazon ½ lb/ton + 1 spray 4 oz/acre on 11/7	3 (15)	52 (152)	26 (39)	0 (0)
Demeton methyl foliage spray - 6 oz/acre x 3 applications on 6/6, 22/6 and 11/7	3 (3)	73 (334)	38 (60)	0 (0)
Control	4 (5)	51 (155)	35 (49)	0 (0)

Note: Aphid observations - First figure gives % infested plants
Bracketed figure gives number of aphids per 300 leaves

Table 6. FOLKINGHAM - Strip Trial - 1961
Planted 14th March 1961 : Variety - Arran Pilot

Treatment (All rates refer to active ingredient)	Aphid observations at various dates				
	25/4	17/5	6/6	22/6	12/7
Tuber treatment - Menazon 1 lb/ton at planting	0	0	3	45 (165)	100 (430)
Tuber treatment - Menazon ½ lb/ton at planting	0	0	5	55 (315)	100 (1117)
Tuber treatment - Menazon 1 lb/ton applied in chitting house on 15th November 1960	0	0	10	63 (654)	100 (558)
Control	0	0	22	96 (3860)	100 (4449)

Note: Aphid observations - First figure gives % infested plants
Bracketed figure gives number of aphids per 300 leaves

Table 7. THREEKINGHAM - Strip Trial - 1961
Planted 9th March 1961 : Variety - Arran Pilot

Treatment (All rates refer to active ingredient)	Aphid observations at various dates			
	25/4	17/5	6/6	23/6
Tuber treatment - Menazon 1 lb/ton at planting	0	0	15	90 (277)
Granule treatment - Menazon granules in rows at planting at rate of 1 lb/acre	0	1	44	96 (674)
Tuber treatment - Menazon 1 lb/ton applied in chitting house on 16th November 1960	0	1	31	98 (527)
Control	0	3	36	100 (1039)

Note: Aphid observations - First figure gives % infested plants
Bracketed figure gives number of aphids per 300 leaves

Table 8. GRAZELEY GREEN - Strip Trial - 1961
Planted 12th/13th May 1961 :
Variety - Majestic

Treatment	Aphid observations at various dates			
(All rates refer to active ingredient)	13/6	16/6	4/7	18/7
Tuber treatment - Menazon 1 lb/ton	5	31 (71)	40 (85)	12.5 (15)
Tuber treatment - Menazon ½ lb/ton	15	38 (84)	65 (192)	25 (30)
Granule treatment - Menazon granules in rows at planting at 2 lb/acre	30	37 (70)	50 (77)	30 (48)
Control	65	74 (442)	80 (260)	25 (48)

Note: Aphid observations - First figure gives % infested plants
Bracketed figure gives numbers of aphids per 300 leaves

Virus disease spread in 1960 Fernhurst trial

Squares show 1960 layout. Figures show numbers of virus infected plants, secondary infections, appearing in plants from 300 tubers sampled from plots and grown on in 1961.

Table 9
Leaf Roll

16 A	17 B	17 C	8 D	2 E
11 C	3 D	9 E	5 A	7 B
16 B	11 C	7 A	6 E	9 D
25 E	14 A	3 D	2 B	13 C
16 D	9 E	2 B	6 C	10 A

Treatments as in Table 11

Table 10

Virus Y

4 A	3 B	1 C	1 D	1 E
8 C	4 D	0 E	2 A	3 B
0 B	2 C	2 A	1 E	0 D
2 E	0 A	1 D	3 B	9 C
2 D	1 E	1 B	0 C	7 A

Table 11

Treatments	Mean % disease	
	Leaf Roll	Virus Y
A Menazon foliage spray - 4 oz/acre x 2 applications	3.3	0.7
B Demeton - methyl foliage spray - 6 oz/acre x 3 applications	2.5	0.5
C Control - unsprayed	3.8	0.9
D Menazon tuber treatment - 1 lb/ton, followed by 1 Menazon foliage spray at 4 oz/acre	2.4	0.4
E Menazon foliage spray - 4 oz/acre x 3 applications	3.0	0.3
Significant difference between treatment means	Not significant	Not significant

EXPERIENCES WITH 5-AMINO-3-PHENYL-1-BIS(DIMETHYLAMIDO) PHOSPHORYL-1,2,4-TRIAZOLE, A NEW FUNGICIDE CONTROLLING POWDERY MILDEW*

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Summary

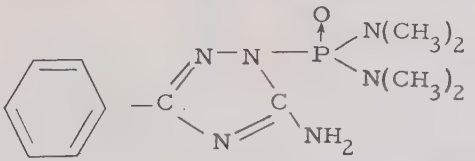
A review of the results with 5-amino-3-phenyl-1-bis(dimethylamido) phosphoryl-1,2,4-triazole is given. This compound, indicated as WP 155, when applied as a leaf spray, has a considerable effect on powdery mildew infection. WP 155 is a poisonous substance with cholinesterase-inhibiting properties.

In laboratory tests a systemic fungicidal effect was demonstrated by applying the substance to the roots of plants. In a number of countries WP 155 is now applied in practice for the control of powdery mildew on a number of ornamental plants, such as roses, Crataegus, Acer, Begonia and Cineraria. Its use on apples is promising and tests are being made on a number of other crop plants.

Apart from fungicidal properties WP 155 also has, under practical conditions, a useful effect on aphids, and some effect on spider mites. The activity against other insects is still under investigation. Honey bees are fairly resistant.

Introduction

This paper presents a review of the results obtained to date by the author and his colleagues with 5-amino-3-phenyl-1-bis(dimethylamido) phosphoryl-1,2,4-triazole. This compound will for convenience be designated WP 155, which was the code number generally used during the investigations. WP 155 is one of a series of amino-triazole derivatives with fungicidal, insecticidal and acaricidal properties described by v.d. Bos, Koopmans and Huisman (1960). Its chemical structure is (v.d. Bos, 1960):



* Communication no. 64 of the Agrobiological Laboratory "Boekesteyn".

The melting point of the pure compound is 167° - 168°. It is soluble in most organic solvents, but only slightly in water. The compound is stable under neutral and basic conditions and rather unstable in the presence of strong mineral acids.

WP 155 has, as is common with other organophosphate insecticides, cholinesterase-inhibiting properties. The acute oral LD 50 for female albino mice appeared to be 20 mg/kg, and the corresponding value for male mice was 32 mg/kg. After intraperitoneal administration, values for the acude LD 50 of 8 and 9 mg/kg for female and male albino mice respectively were found. The acute dermal LD 50 for rabbits lies between 1500 and 3000 mg/kg. WP 155, applied to the skin of rabbits for 20 days in dosages of up to 10 mg/kg, did not cause any mortality, nor any differences with respect to behaviour, food consumption or weight. These dosages caused some, probably reversible, cholinesterase inhibition. Scarification of the skin did not influence the results in this experiment. Cumulative feeding studies are in progress.

Fungicidal activity

In laboratory trials, WP 155 and a number of related compounds, when sprayed on the leaves, gives good protection to plants against attack by members of the Erisyphaceae. As far as is known at present, there are differences in the degree of activity of, but no principal differences in, the type of action between these substances, which, surprisingly, are able to protect the plant against powdery mildew infection when they were administered to the roots (Koopmans, 1960).

An illustration of this systemic effect is given in Table 1.

Table 1. Infection by Podosphaera leucotricha of apple seedlings in WP 155 solutions.

Initial concentr. p.p.m.	Average infection after 14 days		Number of newly formed leaves per plant
	On existing leaves in % of untreated leaves	On newly formed leaves Evaluation of infection *	
31.5	5	0.3	1.1
10	17	1.1	2.3
0	100	2.8	1.5

* 0 = no infection; 1 = light infection; 2 = moderate infection; 3 = severe infection.

The exact mode of the systemic action is not known. Koopmans observed well developed germination tubes with an appressorium after the

application of considerable overdosages of WP 155 that completely arrested any mycelial development. Probably the development can only be arrested when a penetration hypha has been formed and - if this is true - the point of action is obviously situated in the epidermal cells. Treatment of a grape leaf with WP 155 does not result in protection of neighbouring leaves against powdery mildew infection, when the treated leaf is undamaged, but if the leaf is bruised before treatment, then no powdery mildew develops on neighbouring leaves.

Since 1957 field experiments have been carried out with WP 155. On apples a wettable powder containing 25% of active ingredient and an 8.9% water-miscible solution were used. It appeared that the fungicidal effect of WP 155 against apple powdery mildew under practical conditions was the same or slightly better than the effect of dinocap when applied in equal dosages.

The standard concentration for WP 155 is at present considered in the Netherlands to be 0.025% active ingredient, the concentration for dinocap being 0.03%. The usual spray interval with these concentrations has to be some 12 days. Shorter spray intervals - about 1 week with half the normal concentration - usually result in better control and quite a number of growers favour this practice with dinocap despite the increased spraying costs. On apples one can often observe that treatments with WP 155 cause shoots - grown from infected buds and normally completely covered by powdery mildew - to produce healthy top leaves again. Experience so far is that on apples, the interval between WP 155 applications should not be shorter than those employed for sulphur and dinocap, the older mildew control materials.

In most of the trials 25 shoots from each plot were chosen at random and marked. The degree of attack was assessed for each leaf on these shoots. There were as a rule three replications. The assessment was repeated in the course of the growing season on the same shoots. The leaves were graded into four classes: (0) free from mildew lesions; (1) slight attack: one or two small lesions; (3) moderate attack: more than one or two lesions, but less than half the leaf area covered with mycelium; (5) heavy attack: more than 50% of the leaf area covered with mycelium. The degree of attack was obtained by multiplying the percentages of slightly, moderately and heavily infected leaves with the arbitrarily chosen factors 1, 3 and 5 respectively and adding the products. The degree of control achieved by the treatments, as compared to the untreated plots, seems only moderate. This is mainly because the sprays had sometimes to be timed too far apart and pruning, which is a very valuable additional control measure, was omitted.

Table 2. Variety Jonathan. Treatments: 30/4, 9/5, 20/5, 3/6, 14/6.

	% infected leaves						Degree of attack	
	17/6			4/7			17/6	4/7
	light	moderate	heavy	light	moderate	heavy		
WP 155 25% w.p. 0.1%	9	0	0	21	0	0	9	21
dinocap 25% w.p. 0.12%	14	1	1	21	1	3	22	39
Untreated	45	5	1	48	8	2	65	82

Table 3. Variety Jonathan. Treatments: 24/4, 4/5, 16/5, 29/5 10/6, 20/6, 4/7, 16/7, 1/8, 15/8.

	% infected leaves												Degree of attack			
	3/6			18/6			17/7			20/8						
	l	m	h	l	m	h	l	m	h	l	m	h	3/6	18/6	17/7	20/8
WP 155 25% w.p. 0.1%	13	0	0	31	1	0	31	1	1	50	4	2	13	34	39	72
dinocap 25% w.p. 0.12%	10	0	0	23	2	0	27	2	0	48	3	2	10	29	33	67
Untreated	33	1	0	44	4	2	40	5	6	55	8	9	36	66	85	124

Table 4. Variety Lombarts Calville. Treatments: 14/4, 25/4, 16/5, 24/5, 8/6, 20/6, 12/7, 31/7, 18/8.

	% infected leaves						Degree of attack	
	13/6			5/7			13/6	5/7
	light	moderate	heavy	light	moderate	heavy		
WP 155 8.9% e.c. 0.25%	8	1	1	11	0	0	16	11
dinocap 25% w.p. 0.12%	10	1	1	14	1	0	18	17
Untreated	37	6	5	34	9	3	80	76

Table 5. Variety Jonathan. Treatments: 4/5, 13/5, 25/5, 6/6, 12/6, 24/6, 6/7.

	% infected leaves									Degree of attack		
	29/5			25/6			13/8					
	l	m	h	l	m	h	l	m	h	29/5	25/6	13/8
WP 155 25% w.p. 0.1%	10	1	0	35	8	2	41	11	13	13	69	139
dinocap 25% w.p. 0.12%	14	1	1	39	11	4	45	9	13	22	92	137

Table 6. Variety Cox' Orange Pippin. Treatments: 24/4, 13/5, 5/6, 7/7, 24/7.

	% infected leaves						Degree of attack	
	8/7			25/7				
	light	moderate	heavy	light	moderate	heavy	8/7	25/7
WP 155 25% w.p. 0.12%	30	7	4	34	11	7	71	102
dinocap 25% w.p. 0.12%	36	8	3	39	11	4	75	92
Untreated	40	14	12	33	16	17	142	166

Table 7 gives the results of an experiment in four replications in France. Here the leaves were graded in the same way as described above, but the factors 1, 2 and 3 respectively were chosen by the investigator. The results are expressed as the mean of these values for all the leaves of a treatment. 20 shoots were examined per plot.

Table 7. Variety Jonathan. Treatments: 2/4, 16/4, 30/4, 14/5, 11/6, 27/6, 14/7, 1/8.

	Degree of attack	
	17/5	6/7
WP 155 8.9% e.c. 0.25%	0.51	0.18
dinocap 25% w.p. 0.3%	0.90	0.63
Untreated	1.38	1.02

Attempts to control apple powdery mildew by administering WP 155 to the trunks and roots of the trees were made but these treatments were generally too phytotoxic.

Damage to apple leaves has been observed a number of times in the Netherlands but in other countries phytotoxicity has seldom been reported. The injury manifested itself by a narrow yellowing band along the edges of part of the leaves, usually the older ones. In the few more severe cases this extended into a slight irregular yellowing over the whole leaf area or sometimes a necrosis of the leaf edges. These symptoms occurred particularly on the Jonathan variety, and then only after three or more treatments in spring. There are also numerous instances where the normal or even higher concentrations (e.g. 0.2% of the 25% w.p.) gave no discernible symptoms under otherwise similar conditions. Perhaps the growth conditions of the tree or climatic conditions are of importance, but up to now there is no evidence to support these assumptions. Thus phytotoxicity may well restrict the use of WP 155 on Jonathan in the Netherlands. With Golden Delicious marked increases in yield and quality are often noticed, as shown in Table 8.

Table 8. Variety Golden Delicious
Treatments: 1 & 2 : 28/4, 12/5, 29/5, 7/6, 21/6, 3/7, 15/7, 29/7, 14/8.
No. 3 on same days and in addition on 6/5, 18/5, 31/5, 15/6, 28/6, 10/7, 22/7, 7/8.

	kg/150 trees	% of fruits		
		glossy	light russet	russet
WP 155 25% w.p. 0.1%	3400	74	3	23
WP 155 8.9% e.c. 0.25%	3980	59	7	34
dinocap 25% w.p. 0.06%	2620	16	26	58

Scab control with captan on same dates as 1 & 2.

Residue studies on apple fruit are in progress. The results so far indicate a waiting period of 4 to 6 weeks between last treatment and harvest. On average the residues on apple fruits decrease rapidly after a few days and are less than 0.1 p.p.m. within about three weeks or less.

On roses, both greenhouse and outdoor, WP 155 is highly effective against powdery mildew. Treatment with WP 155 as a water-miscible solution (0.2 - 0.25% concentration) is now an accepted practice among rose growers in the Netherlands, Belgium and West-Germany. The treatments appear to be quite safe even on sensitive varieties like Baccarat. The wettable powder cannot be used on roses grown for flowers, because of the disfiguring residue, and in any case it is also somewhat less effective than the liquid formulation. Curative effects have been reported by Betgem (1960). A remarkable shoot growth of roses is

often seen after WP 155 treatment in contrast to the results obtained after dusting with, or vaporization of, sulfur.

Other plants on which powdery mildew can effectively be controlled with WP 155 include Crataegus spp., Acer spp., Begonia, Chrysamthemum and Cineraria. In several countries it is at present being used for these crops. On other plants, including cucumber, delphinium and oaks, the effect of WP 155 against powdery mildew is considerable, but not quite good enough to compete with existing materials.

Tables 9, 10 and 11 illustrate the results obtained in roses and Crataegus.

Table 9. Rose: Variety Nymph. Treatment: 7/7.

	Mean evaluation mildew infection* on 16/7
WP 155 8.9% e.c. 0.25%	8
WP 155 8.9% e.c. 0.5%	9
dinocap 25% w.p. 0.1%	7½
Untreated	7

* 10 = no infection
0 = maximal infection

Table 10. Crataegus monogyna.
Treatments: 17/6, 28/6, 11/7, 20/7, 3/8, 19/8.

	Mean evaluation mildew infection				
	7/7	11/7	3/8	19/8	20/9
WP 155 8.9% e.c. 0.15%	6.3	4.6	6.3	7.3	7.6
WP 155 8.9% e.c. 0.2%	7.0	7.0	7.6	7.6	8.3
WP 155 8.9% e.c. 0.25%	8.3	7.0	7.6	8.6	8.6
dinocap 25% w.p. 0.1%	8.0	6.0	7.3	7.0	8.6
Untreated	4.6	3.6	4.0	5.5	4.6

Table 11. Crataegus monogyna.
Treatments: 20/6, 28/6, 10/7, 26/7, 3/8, 30/8, 12/9.

	% mildew-free shoots	
	19/9	29/9
Dinitro-sec.butylphenyl dimethylacrylate 25% w.p. 0.05%	23	31
WP 155 8.9% e.c. 0.25%	44	41
Untreated	18	23

Insecticidal and acaricidal activity

Meltzer (in the press) described the insecticidal properties of this group of compounds in general, and of WP 155 in particular. WP 155 has considerable contact and systemic aphicidal and acaricidal activity. The systemic effect, however, has only been investigated after the application of WP 155 to the roots of plants. WP 155 was found to have no activity towards the common housefly, whether by contact or in the vapour phase. Honey bees were able to tolerate WP 155 in a concentration of 10 p.p.m. without harmful consequences; 100 p.p.m., however, was lethal. Under practical conditions the effect of WP 155 on aphids, at concentrations required to control mildew, is sometimes satisfactory after a single spray and is in any case adequate if treatment against mildew is given regularly. The effect on spider mites is generally less pronounced, although definitely present. Table 12 represents a case in which the effect on an unspecified spider mite on strawberries was obvious.

Table 12. Strawberries, variety Deutsch Evern.
Treatments: 21/5, 30/5, 14/6.

	Number of spider mites per 100 leaves	
	11/6	17/6
WP 155 25% w.p. 0.1%	208	114
WP 155 25% w.p. 0.2%	86	96
dinocap 25% w.p. 0.1%	684	420
Control	444	964

There are indications that on apples the activity of WP 155 on capsids and sawflies is of practical importance. The effect on woolly aphid in the concentration used against mildew is probably negligible.

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TRIPHENYL TIN HYDROXIDE, A FUNGICIDE FOR THE CONTROL OF PHYTOPHTHORA INFESTANS ON POTATOES, AND SOME OTHER FUNGUS DISEASES

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Summary

A survey is given of laboratory investigations into the fungicidal activity of some organotin compounds. Results are given of field trials with some representatives of the triphenyl tin compounds, especially for the control of Phytophthora infestans in potatoes. Excellent results were obtained particularly with formulations of triphenyl tin hydroxide. Favourable results were also obtained in the control of Cercospora beticola on sugar beets. Results of tests against Phytophthora infestans and Cladosporium fulvum on tomatoes are promising.

In 1950 the Institute for Organic Chemistry, T.N.O. at Utrecht, was requested by the International Tin Research Council in London to carry out scientific and field investigations in the field of organotin compounds. van der Kerk and Luijten (1954) described the high fungicidal activity in vitro of alkyl tin compounds. The fungicidal activity appeared to increase in this direction:- $RSnX_3 \longrightarrow R_2SnX_2 \longrightarrow R_4Sn \longrightarrow R_3SnX$

Table 1. Influence of the number of alkyl groups directly attached to the tin atom on the fungicidal properties of tehyl tin compounds.

		Minimum inhibiting conc. (p.p.m.)			
		Botrytis allii	Penicillium italicum	Aspergillus niger	Rhizopus nigricans
Tetraethyltin	$(C_2H_5)Sn$	50	>1000	100	100
Triethyltin chloride	$(C_2H_5)_3SnCl$	0.5	2	5	2
Diethyltin dichloride	$(C_2H_5)_2SnCl_2$	100	100	500	200
Ethyltin trichloride	$C_2H_5SnCl_3$	>1000	>1000	>1000	>1000
Stannic chloride	$SnCl_4$	>1000	>1000	>1000	>1000
Stannous chloride	$SnCl_2.2\,H_2O$	>1000	>1000	>1000	>1000

(after Van Der Kerk and Luijten (1954))

Only the triethyl tin chloride appeared to be strongly fungicidal. The main influence on the fungicidal effect is exerted by the number of ethyl groups. The nature of the fourth group is of less importance in the triakyl tin compounds for the chloride, sulphate, hydroxide, acetate, etc. showed all practically the same activity. Variation of the alkyl group yielded the results of Table 2, also taken from the publication by van der Kerk and Luijten.

Table 2. Influence of the nature of the group R on the fungicidal properties of compounds $R_3Sn0.C0.Ch_3$

	Minimum inhibiting concentration (p.p.m.)			
	Botrytis allii	Penicillium italicum	Aspergillus niger	Rhizopus nigricans
Trimethyltin acetate	20	20	200	200
Triethyltin acetate	1	2	5	2
Tri-n-propyltin acetate	0.1	0.1	1	1
Triisopropyltin acetate	0.1	0.1	1	0.5
Tri-n-butyltin acetate	0.1	0.1	0.5	0.5
Tri-n-hexyltin acetate	1	10	20	100
Tri-n-octyltin acetate	>100	>100	>100	>100
Triphenyltin acetate	2	1	0.5	10
Phenylmercuric acetate	0.5	0.5	0.5	5

The order of increasing fungicidal activity appeared to be as follows: octyl $\longrightarrow$ methyl $\longrightarrow$ ethyl $\longrightarrow$ propyl $\approx$ butyl.

For the sake of comparison, the well-known mercury fungicide phenyl mercuric acetate was included in the tests.

It is clear that, in the group of the trialkyl compounds, the effect of the tripropyl, triisopropyl and tributyl compounds is sufficient to place the products in the category of powerful fungicides. Though these fungicides may be considered for industrial application, the investigations regarding their effect on agricultural crops have led to disappointment. At concentrations required to control the disease, all the above mentioned fungitoxic trialkyl compounds appeared to be too phytotoxic.

As seen from Table 2, the triaryl compound, triphenyl tin acetate, has a fungicidal effect which is 10 to 20 times less than that of the best trialkyl compounds, but this substance could nevertheless still be looked upon as a powerful fungicide. Tests revealed that this substance is well tolerated by many plants, even in concentrations considerably higher than those required for fungus control.

The high fungicidal activity of the trialkyl compounds in vitro was not revealed under field conditions whereas the triaryl tin compounds, appeared to be powerful fungicides under these conditions. As a consequence particular attention was paid to the triphenyl tin compounds. It soon became clear that the X in the general formula R_3SnX exerted a considerable influence, if not on the fungitoxic, then on the phytotoxic characteristics of the product, which deferred the use of several compounds. On the basis of laboratory experiments, the following sequence of decreasing phytotoxicity was found:- triphenyl tin acetate, triphenyl tin hydroxide, triphenyl tin p-toluene sulfonamide. Fungitoxic tests on Fusarium culmorum, P. infestans and Venturia inaequalis led to this sequence of increasing activity:- triphenyl tin oxalate, triphenyl tin hydroxide, triphenyl tin acetate. On the basis of these and other data, obtained in the laboratory, it was decided to arrange larger field trials with triphenyl tin acetate, triphenyl tin oxalate, triphenyl tin hydroxide and triphenyl tin p-toluene sulfonamide.

All these compounds gave a better control of P. infestans on potato tubers than copper oxychloride. The leaf infestation was always better controlled by the tin compounds than by copper oxychloride, though the differences were smaller towards the end of the season than at the beginning.

To illustrate the point, results are given of two trials on potatoes (Tables 3 and 4). In Table 3 the various preparations were sprayed at identical dosages. A total of 6 spraying was carried out between June 24 and August 27. The seasonal averages of the leaf infestation were based on 6 observations between August 14 and September 6. The scale used was: 10 = free from Phytophthora, and 0 = completely dead.

After statistical analysis it appears that triphenyl tin acetate, and triphenyl tin hydroxide yielded the best control, but that their mutual differences were statistically not significant at a level $P = 0.05$. Triphenyl tin oxalate, copper oxychloride and triphenyl tin p-toluene sulfonamide, though statistically highly significantly better at a level $P = 0.01$ than untreated lagged behind the products of the first-mentioned group.

With reference to phytotoxicity, 10 stands for "no injury" and 0 = completely dead. Much injury, particularly in the later growth occurred with triphenyl tin acetate. Only slight injury was caused by the other compounds. Triphenyl tin p-toluene sulfonamide and triphenyl tin hydroxide were the safest and even better than copper oxychloride.

Practically all treatments led to a higher yield of healthy tubers than copper oxychloride. However, attention should be drawn particularly to the percentage of tubers infected by Phytophthora. In all the tin treatments, except triphenyl tin p-toluene sulfonamide, the percentage of infected tubers is significantly lower than with copper oxychloride.

Table 3. Niew Vennep (clay, variety Bintje)

	Spraying dates and dosages in kg/ha							Seasonal averages leaf infestation 1)		Average injury figures 3)		Average yield of 50 plants			
	24/6	3/7	15/7	25/7	6/8	18/8	27/8		Transf. 2)	18/8	Transf. 2)	Healthy in kg	copper oxychl. = 100	% diseased	Transf. 2)
1. Untreated								2.4	2.75	10	5.74	40.2	81	19.9	26.41
2. Copper oxychloride 50%	7	8	9	10	10	10	10	5.6	4.29	8.1	5.15	49.7	=100	8.6	16.80
3. TPT-acetate 20% w.p.	1.8	2.0	2.2	2.4	2.4	2.4	2.4	7.4	4.94	6.6	4.64	53.1	107	2.6	8.75
4. TPT-oxalate 20% w.p.	1.8	2.0	2.2	2.4	2.4	2.4	2.4	6.1	4.46	8.3	5.20	53.4	107	4.9	12.67
5. TPT-hydroxide 20% w.p.	1.8	2.0	2.2	2.4	2.4	2.4	2.4	7.1	4.84	9.4	5.55	52.4	105	3.1	10.03
6. TPT-p-toluene sulfonamide 20% w.p.	1.8	2.0	2.2	2.4	2.4	2.4	2.4	4.4	3.76	9.6	5.61	49.4	99	11.9	19.95
P 0.05									0.49		0.31	4.7			4.64
P 0.01									0.74		0.47	7.2			7.03

1) 10 = free from Phytophthora; 0 = completely dead.
2) Transformation applied: bg sin $\sqrt{\frac{x}{10}}$
3) 10 = no injury.

Table 4. Boekesteyn (sand, variety Bintje)

	Spraying dates and dosages in kg/ha on:						Seasonal averages leaf infestation 1)		Average injury figures 3)		Average yield of 50 plants			
	16/6	26/6	4/7	15/7	25/7	13/8		Transf. 2)	30/7	Transf. 2)	Healthy in kg	copper oxychl. = 100	% diseased	Transf.
1. Untreated							2.8	3.02	0	0.40	43.9	108	3.6	10.63
2. Copper oxychloride 50%	7	8	9	10	11	11	5.6	4.31	0.9	3.46	40.7	=100	10.6	18.59
3. TPT-acetate 20% w. p.	1.8	2.0	2.2	2.4	2.4	2.4	6.3	4.55	2.9	5.93	42.5	104	4.4	12.19
4. TPT-oxalate 20% w. p.	1.8	2.0	2.2	2.4	2.4	2.4	5.2	4.15	1.1	3.78	45.4	112	3.4	10.75
5. TPT-hydroxide 20% w. p.	1.8	2.0	2.2	2.4	2.4	2.4	6.4	4.59	1.8	4.55	44.6	110	6.2	14.49
6. TPT p-toluene sulfonamide 20% w. p.	1.8	2.0	2.2	2.4	2.4	2.4	4.0	3.61	0.3	1.09	42.5	104	4.5	12.37
P 0.05								0.34		0.96				3.55
P 0.01								0.51		1.45	N.S.4)			5.38

1) 10 = free from Phytophthora; 0 = completely dead
2) applied transformation: bg $\sin\sqrt{x}$
3) 0 = no injury; 1, 2, 3 = light, moderate and heavy injury, respectively.
4) N.S. = Statistically not significant.

It appears, from Table 4, that the control of Phytophthora in the haulm obtained by the hydroxide and the acetate was better than that obtained by copper oxychloride, though the differences were statistically not significant. In this table phytotoxicity is expressed by the following figures: 0 = no injury; 1 = slight injury; 2 = moderate injury; 3 = heavy injury. The injury was lowest for triphenyl tin p-toluene sulfonamide; then came copper oxychloride, triphenyl tin oxalate, triphenyl tin hydroxide. Triphenyl tin acetate produced very serious injury. As compared with copper oxychloride, all the treatments gave higher yields; the best in this respect were triphenyl tin oxalate and triphenyl tin hydroxide. The percentage of diseased tubers was in all the tin treatments significantly lower than with copper oxychloride.

In view of the results obtained, it was decided to continue the field investigations mainly with triphenyl tin hydroxide and triphenyl tin acetate. Unfortunately, however, very little Phytophthora occurred the next year, so that little information was obtained on the control of the disease. Important data were obtained, on the other hand, on phytotoxicity, as shown in Table 5.

Table 5

	Dosage in kg/ha	Average injury figure on 14.8
Untreated	-	0
Copper oxychloride	7 - 10	0
Triphenyl tin acetate w.p. 20%	1.2 - 1.5	1.3
Triphenyl tin hydroxide w.p. 20%	1.2 - 1.5	0

Figures: 0 = no injury; 1 = slight injury; 2 = moderate injury; 3 = heavy injury

It had meanwhile been found that the degree of phytotoxicity of the tin compounds could be influenced to a considerable extent by the formulation. It became evident, for instance, that besides the influence the particle size of the active substance has on the biological behaviour, the addition of certain organic colloids to the formulation can reduce the phytotoxicity, pH also appeared to exert a considerable influence. The application of this knowledge to new formulations led to improvements in the field results; though favourable fungitoxic properties were maintained, the phytotoxicity was reduced.

Once again it appeared that on potatoes, the hydroxide was better than the acetate. Some injury was still caused by the new acetate formulation but the triphenyl tin hydroxide formulation gave hardly any injury. Table 6

illustrates the effect of triphenyl tin hydroxide in various dosages compared with, amongst others, triphenyl tin acetate. If triphenyl tin hydroxide and triphenyl tin acetate are compared at similar dosages, it will be seen that the seasonal average of the Phytophthora infestation for triphenyl tin hydroxide was 5.6 and for triphenyl tin acetate 5.3. For this evaluation a scale in which 0 = heavy infestation and 10 = no infestation, was used.

The scale for injury runs from 0 = no injury to 5 = serious injury. That caused by triphenyl tin hydroxide in this trial was 0.7 against 1.5 for the acetate. All the tin treatments gave higher yields of healthy tubers than the untreated.

Table 7 shows that triphenyl tin hydroxide controlled the leaf infestation better than triphenyl tin acetate, and that the net tuber yield and the percentage of diseased tubers was lower with triphenyl tin hydroxide than with triphenyl tin acetate.

Table 6. Boekesteyn (sand, variety Bintje)

	Spraying dates and dosages in kg prep. per ha. on:				Seasonal averages leaf infestation 1)	Transf. 2)	Average injury figures 3)	Average yield of 50 plants in tubers >30 mm.	
	15/6	11/7	27/7	4/8				Healthy in kg	% diseased
Untreated					3.2	34.8	1.0	32	12.8
Triphenyl tin acetate w.p.20%	1.5	1.6	1.7	1.8	5.3	46.1	1.5	34	13.8
Triphenyl tin hydroxide w.p.20%	1.5	1.6	1.7	1.8	5.6	49.4	0.7	34	13.0
Triphenyl tin hydroxide w.p.20%	1.8	2.0	2.2	2.4	5.8	50.1	0.8	38	13.2
Triphenyl tin chloride w.p.20%	1.5	1.6	1.7	1.8	5.4	47.6		34	15.9
P 0.05						6.3		N.S.	N.S.
P 0.01						9.5			

1) 10 = free from Phytophthora infestans; 0 = completely dead
2) Transformation: bg sin $\sqrt{10x}$
3) 0 = injury
1, 2, 3 = light, moderate and heavy injury, respectively.

Table 7. Boekesteyn (sand, variety Bintje)

	Spraying dates and dosages in kg prep. per ha on:				Seasonal averages leaf infestation 1)	Transf. 2)	Average injury figures 3)	Average yield of 50 plants in tubers >30 mm.	
	15/6	11/7	26/7	4/8				Healthy in kg	% diseased
Untreated					2.8	31.6	0.8	42	9.1
Triphenyl tin acetate w.p. 20%	1.5	1.6	1.7	1.8	4.7	43.6	1.3	36	15
Triphenyl tin hydroxide w.p. 20%	1.5	1.6	1.7	1.8	5.1	46.6	1.3	39	12.5
Triphenyl tin chloride w.p. 20%	1.5	1.6	1.7	1.8	4.9	44.5	1.5	41	11.5
P 0.05						1.7		N.S.	N.S.
P 0.01						2.6			

1) 10 = free from Phytophthora infestans; 0 = completely dead
2) Transformation bg sin $\sqrt{10x}$
3) 0 = no injury. 1, 2, 3 light, moderate and heavy injury, respectively.

Finally a few remarks on uses other than on potatoes. On celeriac, triphenyl tin hydroxide in 0.3% appeared to give a good control of Septoria apii. On beets, Cercospora can be controlled effectively with triphenyl tin hydroxide. The dosage in this application was taken at the same level as that applied on potatoes, but will be further investigated.

Encouraging results were obtained on tomatoes in the control of P. infestans and Cladosporium fulvum. Injury occasionally occurred with the old formulations, but the new formulations of triphenyl tin hydroxide were much better in this respect. On apples complete control of scab was obtained, even with a small number of sprayings. Although some phytotoxicity occurred, the triphenyl tin hydroxide formulation approved to be safer than triphenyl tin acetate. Table 8 provides some data on results with triphenyl tin compounds on apples.

Table 8. 's-Graveland, Golden Delicious

	Dosages	Fruit russeting	Yields in kg
Triphenyl tin acetate w.p. 20%	0.1 - 0.05%	6	1500
Triphenyl tin hydroxide w.p. 20%	0.1 - 0.05%	6.5	1500
captan w.p.	0.25 - 0.125%	8	1800
P 0.01			554
P 0.05			366

Evaluation figures for fruit russeting: 1 = complete russeting
10 = no fruit russeting at all.

The quality of the apples treated with triphenyl tin hydroxide and triphenyl tin acetate was below that of the fruit treated with captan.

In another trial on apples, triphenyl tin hydroxide produced a higher yield than triphenyl tin acetate and captan, but the russeting of the apples was considerable (Table 9).

Table 9. Sevenum, Golden Delicious

	Yield in kg. per 3 trees
Triphenyl tin acetate w.p.	436
Triphenyl tin hydroxide w.p.	552
captan w.p.	480

A further investigation with the triphenyl tin hydroxide formulation, which has meanwhile been marketed for potatoes, beets and celery, is being made on various crops and in regions with different climates.

Finally the following chemical, physical and toxicological data are given:

Triphenyl tin hydroxide

Gross formula: $C_{18}H_{16}O\,Sn$
Molecular weight: 366.7
Appearance: white crystalline substance; Melting point: 118 - 120° C. (If the substance is slowly heated, water is evolved)
Solubility: in ether at 20° C. 28 gm/l; 1.2 dichlorethane: 74 gm/l; methylene chloride: 171 gm/l; benzene: 41 gm/l; ethanol: abt. 100 gm/l; practically insoluble in water.

Acute toxicity in mice in mg/kg with 95% confidence limits.

		oral	intraperitoneal
LD 50	♀	511 (243 - 1080)	10.8 (6.65 - 17.5)
		619 (342 - 1120)	17.1 (12.6 - 23.3)

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PRELIMINARY RESULTS WITH A NEW COMPOUND FOR THE CONTROL OF APPLE MILDEW AND RED SPIDER IN THE U.K.

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Summary

HOE 2784 (2-s-butyl-4, 6-dinitrophenyl 3, 3-dimethylacrylate) has been found in Germany to be active against both apple mildew and red spider. Work on red spider in progress for the past two years in the U.K. and on apple mildew for the past year, and the compound has been found to be extremely effective for the control of apple mildew. When used in a programme of sprays every 14 days for the control of mildew, the control of red spider has been good and it has not been found necessary to include any other acaricide in the spray programme. When used as an acaricide alone in a programme of only two sprays the control of Fruit Tree Red Spider mite has been poor under U.K. conditions, and although more are required to give adequate control of this pest, it may not require the six or seven applications of a mildew programme.

Results are presented to illustrate the activity of the compound against both red spider and mildew and details are given showing how the compound is expected to fit into commercial spray programmes in the U.K.

Introduction

HOE 2784 has been developed in Germany by Farbwerke Hoechst A.G. and is based on the active compound 2-s-butyl-4, 6-dinitrophenyl 3, 3-dimethylacrylate. The compound was first reported by Emmel and Czech (1960) to have good acaricidal properties and in particular to be effective for the control of mites which have become resistant to organo-phosphorus or chlorinated hydrocarbon acaricides. Recently Hartel (in print) reported that the compound was also very effective for the control of true powdery mildews and discussed the relationship of chemical configuration to red spider and powdery mildew activity. In this work he showed that of all the compounds tested, the dimethylacrylate ester of dinoseb had the best all-round activity to spider and mildew with minimum phytotoxicity. In the following paper the general properties of the compound will be briefly reviewed and results presented on the performance of the compound in trials in the U.K. during 1961.

General Properties

The technical material is a white solid with a melting point of 67 - 69°C and a faint aromatic odour. It is insoluble in water but soluble in all the usual organic solvents.

Residues in plant material can be determined by Pietzka's method. The ester is extracted from the plant material with n-heptane and hydrolysed with sodium ethylate. The dinoseb formed is steam-distilled to re-

move interfering plant material and measured colorimetrically in dilute NaOH at a wavelength of 420 mμ

The compound is formulated as a 25% dispersible powder and the method of formulation is extremely critical. Faulty formulation can lead to almost complete deactivation of the active material. It is compatible with all normal fungicides such as captan, zineb and thiram. All dispersible powder formulations of insecticides are compatible apart from those based on organo-phosphorus compounds, with which phytotoxicity has been reported from Europe.

Activity against Powdery Mildews

Exploratory work by Besemer (1961) has shown that there are differences in the susceptibility of individual mildew species to HOE 2784. The compound has poor activity against mildew on roses, oaks and wheat but is particularly effective against apple mildew, and work so far carried out in the U.K. has only been against this disease.

Field Trials

Five field trials on Cox's Orange Pippin were carried out in the U.K. during 1961, three sprayed with hand lances using fully replicated plots and two applied with automatic equipment in unreplicated strips.

The three replicated trials were identical in design and situated in Cambridgeshire and Nottinghamshire. They were based on duplicate tree plots with a minimum of four replicates per treatment. These were applied with hand lances at 100 lb/in. as a drenching spray commencing at Pink Bud and repeating every 14 days as far as the weather permitted, to give a total of seven sprays through the season. Primary infection was assessed by counting infected fruit spurs at Pink Bud and secondary infection by selecting 10 extension shoots at random on each tree. The number of infected and clean leaves are counted and then expressed as a percentage infection. Results are given in Tables 1a, 1b and 1c.

Statistical analysis was carried out by using angular transformation of percentages.

Table 1a. Combined Apple Mildew/Red Spider trial carried out in Cambridgeshire

Treatment	Rate/ 100 gall	% Primary infection at Pink Bud	Mildew Assessment: % infected leaves 14.6.61	% infected leaves 7.7.61	% infected leaves including leaves dropped 27.7.61	% leaf drop 27.7.61
HOE 2784	1 lb	21.3	19.2	32.0	35.3	6.5
HOE 2784	2 lb	19.2	12.7	27.5	21.3	5.5
Dinocap	1 lb	24.1	22.9	28.5	35.3	6.8
Untreated	-	17.9	48.7	53.2	52.2	9.3

HOE 2784 at 2 lb is significantly better than all other treatments on 1st and 3rd assessments ($p = 0.01$).
All treatments significantly better than untreated ($p = 0.01$).
Dates of application:- 18.4.61, 4.5.61, 17.5.61, 31.5.61, 15.6.61, 28.6.61 and 17.7.61 of 25% dispensible powders.

Table 1b. Trial carried out in Nottinghamshire, Site A

Treatment	Rate/ 100 gall	% Primary infection at Pink Bud	Mildew Assessment: % infected leaves 30.5.61	% infected leaves 4.7.61	% infected leaves including leaves dropped 17.8.61	% leaf drop 17.8.61
HOE 2784	1 lb	5.3	8.2	27.2	19.4	2.2
HOE 2784	2 lb	2.3	5.8	12.2	10.6	2.8
Dinocap	1 lb	3.1	14.1	22.2	15.6	2.7
Untreated	-	1.9	22.2	52.4	33.5	6.2

HOE 2784 at 2 lb significantly better than all other treatments ($p = 0.05$); all treatments significantly better than untreated ($p = 0.01$) except dinocap at first assessment (not significant).
Dates of application:- 20.4.61, 5.5.61, 18.5.61, 1.6.61, 16.6.61, 29.6.61.and 18.7.61.

Table 1c. Trial carried out in Nottinghamshire, Site B

Treatment	Rate/ 100 gall	Mildew Assessment: % Primary infection at Pink Bud	% infected leaves 29.5.61	% infected leaves 6.7.61	% infected leaves including leaves dropped 10.8.61	% leaf drop 10.8.61
HOE 2784	1 lb	15.7	8.6	21.3	19.4	2.3
HOE 2784	2 lb	10.1	1.9	16.9	18.7	3.0
Dinocap	1 lb	15.2	8.9	26.5	19.8	2.8
Untreated	-	12.5	30.0	66.0	37.3	6.7

HOE 2784 at 2 lb significantly better than all other treatments on 1st assessment only ($p = 0.01$); all treatments significantly better than untreated ($p = 0.01$).
Dates of application:- 19.4.61, 3.5.61, 17.5.61, 1.6.61, 16.6.61, 30.6.61 and 12.7.61.

These results show that rate for rate, HOE 2784 is as good as dinocap in mildew control. The cost of HOE 2784 permits a higher rate to be used, however, and it is clear that the 2 lb rate is giving a better control. No spray damage have been noted from any of the treatments. The incidence of mildew in all trials has been the highest recorded for some years. Of the two sites in Nottinghamshire, Site A had the lower primary infection but secondary infection built up rapidly as the season progressed. Site B had a heavy primary infection which caused a rapid build-up of secondary infection by the 6th July. Heavy rainfall occurred after this date and the infection receded naturally which explains the lower counts on the third assessment. Primary infection counts from all plots will be made in the spring of 1962.

The two unreplicated trials using automatic equipment on Cox's Orange Pippin were carried out in Nottinghamshire and Essex. One was designed to test the compound at high and medium volumes using a seven spray programme and the other to give a field comparison in one of the worst mildew areas in Essex, where six sprays were applied. The results are given in Tables 2a and 2b.

Table 2a. Unreplicated plots in Nottinghamshire, Site A

Treatment	Rate/ acre	Vol. spray/ acre	Mildew Assessment % Primary infection at Pink Bud	% infected leaves 30.5.61	% infected leaves 4.7.61	% infected leaves 17.8.61	% leaf drop 17.8.61
HOE 2784	4 lb	200 gall	2.5	2.1	5.9	14.5	2.6
HOE 2784	4 lb	50 gall	2.6	1.9	6.1	15.4	2.6
Dinocap	2 lb	200 gall	2.0	1.3	6.6	14.1	3.0
Untreated	-	-	1.8	15.3	33.8	44.4	3.3

Dates of application:- 21.4.61, 8.5.61, 19.5.61, 2.6.61, 16.6.61, 30.6.61 and 14.7.61.

Table 2b. Combined Apple Mildew/Red Spider trial in Essex

Treatment	Rate/ acre	Vol. spray/ acre	% Primary infection at Pink Bud	% infected leaves 13.6.61	% infected leaves 14.7.61	% infected leaves 9.8.61	% leaf drop 9.8.61
HOE 2784	4 lb	200 gall	50.8	19.6	44.0	33.8	9.4
Dinocap	2 lb	200 gall	39.4	29.6	54.5	49.8	21.5

Dates of application:- 20.4.61, 8.5.61, 25.5.61, 16.6.61, 3.7.61 and 19.7.61.

Taking into account the higher primary infection counts on the HOE 2784 treatments, the results confirm under "commercial conditions" the findings in the smaller hand-sprayed trials and indicate that HOE 2784 can be sprayed at either high or medium volume to give the same degree of control of apple mildew.

In the Essex trial (Table 2b) mildew has been a very serious problem for three years and the primary infector counts illustrate this point. The grower concerned was satisfied with the control given by HOE 2784 and the figures suggest that the 'build-up' of mildew has been checked.

Activity against Red Spider Mites

Emmel and Czech (1960) and Lhoste and Lambert (1961) reported that HOE 2784 was active against red spider mites, including the Glasshouse Red Spider Mite and the Fruit Tree Red Spider mite which are of economic importance in the U.K. Laboratory tests have confirmed this activity. However, as damage was seen in tests on tomatoes, the main acaricidal work has been on top fruit and in particular against the Fruit Tree Red Spider mite.

After preliminary work in 1960, five field trials were carried out in 1961. Two of the trials were combined with those for apple mildew so that both the efficiency of the compound in a two spray acaricidal programme and the effect of a six or seven spray mildew programme could be observed. In the acaricidal programme two sprays were applied, the first at Pink Bud when the mites were beginning to hatch from the winter eggs and the second in June with the first codlin spray. The timing of the first application at Pink Bud was based on experience on the Continent. A timing trial carried out in Nottinghamshire in 1961 showed that there was no significant difference ($p = 0.05$) in the control obtained between applications of the compound at Pink Bud or Petal Fall.

As with the Mildew work trials were of the two types, three being replicated plots sprayed with hand lances and the other two unreplicated strips sprayed with automatic machines.

The replicated trials on Cox's Orange Pippin were based on one or two tree plots with at least four replicates per treatment. Red spider was assessed by taking a minimum of 50 leaves per plot and brushing the mites on to cards using a mite brushing machine similar to that described by Henderson and McBurnie (1943). The mites were then counted into one of three stages: adults, nymphs and larvae, and eggs.

Results are given in Tables 3a, 3b and 3c.

Table 3c. Combined Red Spider/Mildew trial in Cambridgeshire

Treatment	Rate/ 100 gall	No. of sprays	Fruit Tree Red Spider assessment (No. of mites/leaf) 7th June, 1961			10th July, 1961			24th Aug. 1961		
			Adults	N & L	Eggs	Adults	N & L	Eggs	Adults	N & L	Eggs
1. HOE 2784	2 lb	7	0.00	0.04	0.32	0.04	0.12	0.56	0.04	0.60	2.00
2. HOE 2784	1 lb	7	0.08	0.12	1.36	0.00	0.00	0.76	0.08	1.56	5.08
3. Dinocap	1 lb	7	0.24	0.60	1.40	0.00	0.40	0.96	0.60	4.52	10.32
4. HOE 2784	2 lb	2*	0.00	0.00	0.52	0.00	0.16	0.80	0.28	2.00	4.64
5. HOE 2784	1 lb	2*	0.04	0.24	1.00	0.00	0.56	0.36	0.60	2.68	7.52
6. Phenkapton	16 fl. oz	2*	0.00	0.00	0.20	0.00	0.04	0.04	0.04	0.48	1.24
7. Untreated	-	0	0.32	0.28	6.80	0.48	2.92	18.84	-	-	-
Least difference required for significance	p = .05		-	-	2.09	-	-	2.96	-	-	4.41
	p = .01		-		2.80	-	-	3.97	-	-	5.91

Dates of application

7 spray treatments, 19.4.61, 3.5.61, 12.5.61, 1.6.61, 16.6.61, 30.6.61 and 12.7.61.
*2 spray treatments, 19.4.61 and 16.6.61.
*Dinocap was applied at 1 lb/100 gallons on treatments 4 and 5 on 3.5.61, 12.5.61, 30.6.61 and 12.7.61 and on all dates on treatment 6.

Table 3b. Trial carried out in Essex

Treatment	Rate/ 100 gall	Fruit Tree Red Spider assessment (No. of mites/leaf) 29th May 1961			6th July 1961			1st August 1961		
		Adults	N & L	Eggs	Adults	N & L	Eggs	Adults	N & L	Eggs
1. HOE 2784	1 lb	1.34	0.38	4.16	0.77	6.27	18.18	5.84	64.80	39.52
2. HOE 2784	2 lb	0.26	1.15	1.60	1.73	2.82	16.51	4.48	67.68	46.56
3. Phenkapton	16 fl. oz	0.06	0.45	0.38	0.13	0.70	4.10	0.64	5.44	5.60
4. Untreated	-	1.98	0.45	6.27	2.18	7.81	32.38	5.20	106.08	68.08
Least difference required for significance	p = .05	-		2.32	-	-	9.17	-	-	16.63
	p = .01	-		3.13	-	-	12.35	-	-	22.40

Dates of application

Treatments 1 and 2 Pink Bud - 17th April, 1961.
Treatments 3 Petal Fall - 9th May, 1961.
All treatments 1st Codlin Spray - 13th June, 1961.

Table 3c. Trial carried out in Nottinghamshire

Treatment	Rate/ 100 gall	Fruit Tree Red Spider assessment (No. of mites/leaf) 20th June, 1961			26th July, 1961		
		Adults	N & L	Eggs	Adults	N & L	Eggs
1. HOE 2784	2 lb	0.40	1.24	17.28	0.36	9.40	12.88
2. HOE 2784	1 lb	0.40	4.56	25.08	1.28	24.92	25.68
3. Phenkapton	16 fl.oz	0.04	0.32	1.72	0.12	1.36	2.16
4. Demeton-methyl	12 fl.oz	0.04	0.64	2.76	0.00	1.44	3.04
5. Untreated	-	0.52	5.72	33.44	2.28	41.80	27.72
Least difference required for significance p = .05		-	-	11.76	-	-	9.84
p = .01		-	-	15.73	-	-	13.13

Dates of application

Treatments 1 and 2 Late Pink Bud - 24th April, 1961.
Treatments 3 and 4 Petal Fall - 10th May, 1961.
All treatments 23rd June, 1961.

These results show that the compound, even at 2 lb/100 gallons, does not give a satisfactory control of Red Spider when applied only twice during the season. When, however, it is applied regularly in a mildew programme, the control is adequate and no additional acaricide should be needed.

Both of the automatic machine trials were in Essex; in one a two spray acaricidal programme using medium and high volume was compared to a six spray mildew programme at high volume. The other trial was to compare at high volume a two spray acaricidal programme with a grower's spray schedule.

As the plots were unreplicated, sampling of red spider was carried out using 10 marked trees per plot. 50 leaves were taken at random from each of the marked trees making a total of 500 per plot. Results are given in Tables 4a and 4b.

Table 4a. Combined Red Spider/Mildew trial in Essex, Site A

Treatment	Rate & volume/acre	No. of sprays	Fruit Tree Red Spider assessment (No. of mites/leaf) 29th May, 1961			6th July, 1961			1st Aug. 1961		
			Adults	N & L	Eggs	Adults	N & L	Eggs	Adults	N & L	Eggs
1. HOE 2784	4 lb in 200 gall	6	0.03	0.48	1.44	0.00	0.19	1.38	0.06	1.06	3.39
2. HOE 2784	4 lb in 200 gall	2	0.16	0.19	0.70	0.10	1.73	4.48	0.96	20.06	17.25
3. HOE 2784	4 lb in 50 gall	2	0.29	0.35	1.06	0.26	2.27	5.70	0.51	14.43	14.82

Variety: Cox's Orange Pippin.
Dates of application: 20.4.61, 8.5.61, 25.5.61, 16.6.61, 3.7.61 and 19.7.61.

Table 4b. Trials carried out in Essex, Site B

Treatment	Rate/100 gall	Fruit Tree Red Spider assessment (No. of mites/leaf) 29th July, 1961			6th July, 1961			1st Aug. 1961		
		Adults	N & L	Eggs	Adults	N & L	Eggs	Adults	N & L	Eggs
1. HOE 2784	2 lb	0.58	19.36	1.28	4.29	22.01	31.36	0.22	17.34	48.10
2. Phosphamidon & malathion	16 fl.oz 30 fl.oz	0.13	23.84	0.10	0.35	5.09	3.55	0.16	2.24	2.56

Variety: Worcester Pearmain.
Dates of application: Treatment 1 23.4.61, 16.6.61, 11.7.61 and 25.7.61.
Treatment 2 Phosphamidon 12.5.61
Malathion 19.5.61, 16.6.61 and 6.7.61.
* Bronzing was apparent on the 6th July in the HOE 2784 plot.

These results confirm those of the hand-sprayed trials that satisfactory control of Red Spider cannot be obtained by using only two sprays of the compound but when applied in a Mildew programme it is excellent. It may not, however, be necessary to apply as many as 6 or 7 sprays to obtain a satisfactory control.

The reasons for the inadequate control of a two spray programme under U.K. conditions appear to be two-fold. First, the persistence of the compound is only about fifteen days and, unless the hatch of mites from the winter eggs is very rapid, its potency will have gone before the hatch has been completed. Secondly the compound is unable to diffuse across the leaf surface and as a complete spray cover cannot usually be achieved, a larger number of mites survive the treatment than occurs with materials able to diffuse. Spraying several times increases the cover and consequently gives adequate control. Temperature may also be a factor as the compound has given excellent control of spider in some European countries.

Safety on apple varieties

A wide range of apples have been sprayed including all the main commercial varieties such as Cox's Orange Pippin, Worcester Pearmain, Lord Lambourne, Laxton Superb, Bramley and James Grieve and no varietal susceptibility has been observed.

Discussion

Even though apple mildew is one of the most difficult problems that growers have to face, many are not yet prepared to apply a seven spray programme of a mildew-specific fungicide because of the cost. The results of using HOE 2784 in such a programme show the excellent control of mildew and red spider obtained, and, as no additional acaricide is needed, it will be economic. Difficulties in controlling red spider are arising where the same acaricides are used too frequently without any rotation. The activity of HOE 2784 against mites resistant to other acaricides offers an alternative programme and will therefore help to prevent the build-up of strains resistant to other materials.

No great difficulty is foreseen in fitting HOE 2784 into commercial spray programmes. Although it may not be possible to use organo-phosphorus insecticides, insect pests can be controlled satisfactorily without phytotoxicity using a programme based on BHC-DDT pre-blossom, BHC at Petal Fall for sawfly and DDT or Sevin for codlin moth and summer tortrix. As the compound does not control apple scab an additional fungicide must be used and captan has proved satisfactory. Mixtures with thiram and zineb have also been used successfully on the Continent.

Conclusions

The results of the trials described show that HOE 2784 gives excellent control of apple mildew and red spider in a six or seven spray programme and will be an important addition to the pesticides available to the grower.

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Discussion

Q. Dr. W. E. Ripper

Menazon is obviously a major advance in selective aphis control and the research workers of Plant Protection Ltd., are to be congratulated. What is the mode of insecticidal action and how is the systemic effect achieved? As the prolonged protective effect is based on spray residues, may we ask whether the spray deposit is reasonably rainfast? Does the seed treatment of beet make the beet plants toxic to aphids long enough to convey protection during the months when aphids appear?

A. Mr. J. F. Newman

In reply to the first part, the mode of action of menazon is indeed an interesting subject on which we have limited information at the moment. With locusts, it has been found that menazon is a rather poor cholinesterase inhibitor. I have done some experiments with cercal reflex preparations of cockroaches and menazon is rather poor as a ganglionic blocking agent. I have also tried with cockroach preparations with the perineural membrane removed and there again menazon has poor activity.

With regard to the rainfastness of the deposit of menazon on the surface of leaves, we have had a certain amount of trouble with a dispersible powder formulation which we used in the early part of the season this year, and which was unsatisfactory on some leaves, especially the waxy leaves of sugar beet. We now have some very much improved formulations which we propose to use on sugar beet next year.

A. Mr. E. C. Edgar also replied to this question

With reference to the part of the question on sugar beet seed treatment, the period of protection depends to some extent on the dosage applied. Laboratory tests using 2.0% menazon by weight on the seed gave complete protection against aphid infestation for 6 weeks from sowing the seed and partial control for a much longer period. Higher loadings are desirable although 4.0% is the highest that can be achieved without employing special techniques involving stickers or pelleting. In one field trial this year a 2.0% w/w menazon seed dressing increased the yield of roots by 16%. This was on a late drilled crop and aphid attack was early in 1961.

A. Mr. J. F. Newman then enlarged further on the Mode of action

There are two possibilities. Either some sort of metabolism takes place in which menazon is converted into a more active inhibitor or menazon is active on an enzyme specific to aphids. These are two possibilities and more work is needed to elucidate the matter.

Q. Dr. Hubert Martin

Is the P_{32} revealed in Mr. de Pietri-Tonelli's extremely good radio autographs present in toxic form?

A. Mr. P. de Pietri-Tonelli

The first and second radio autographs were one of each leaf in order of peach trees and, of course, everything was radioactive when photographed. The black spots mark P32 from the insecticide and from all the other metabolites having P_{32}.

Q. Dr. L. Broadbent

The 1960 replicated trials with menazon were well done, but unfortunately sites were near diseased potato crops from which virus was carried to plants in the trials. Mr. Newman should not conclude from these two trials that menazon will be of value only in moderately satisfactory or marginal seed producing areas. Our spraying trials over several years have shown that a good aphicide will limit virus spread in many ware-growing districts and I would expect that further work will show menazon to be effective in such areas?

A. Mr. J. F. Newman

I think Dr. Broadbent is undoubtedly right and one really has to look at those results particularly in relation to the results on aphid populations. Over the last 10 years Dr. Broadbent, Mr. Burt and Mr. Heathcote have done a large number of experiments on the control of aphid-borne viruses in potatoes and they have shown quite clearly that the spray programme with 3 sprays will give the desired control of virus transmission in many parts of England and Wales. We have been able to show in much more limited experiments that we can get control by the tuber treatment which looks quite as good so far as aphid control is concerned and we suppose that the eventual virus control will also be as good. Farmers have not used the spray treatments to any great extent, due I think to the trouble involved in going through the crop several times. The relatively simple tuber application might be more attractive.

Q. Dr. W. E. Ripper

Are thrips killed by menazon?

A. Mr. J. F Newman

Menazon has low activity against thrips and we are quite as baffled as Dr. Ripper is at the moment.

Q. Dr. L. Broadbent

Has menazon been tried as a seed treatment on cereals and carrots to control virus in these crops?

A. Mr. J. F. Newman

In collaboration with Dr. Morgan Jones of the N.A.A.S., Wales, I did do a few trials on the cereal virus down in Pembroke. Dr. Morgan Jones selected fields which had showed very severe virus symptoms in past years, and we got a good attack of aphids as well, but no virus turned up whatever even in the controls.

Q. Mr. M.J. Way

Has Mr. Newman any information on the toxicity and persistence of menazon applied at the same rate per acre as a tuber treatment and as a granular application. In my experiments the same rate per acre was initially more toxic to aphids and also much more persistent when applied to the seed than when applied to granules to the seed harrow?

A. Mr. J.R. Newman

In the potato trials which I described, the rates were the same - 2 lb per ton in the case of tuber treatment and 2 lb per acre in the case of the granule treatment and planting was at the rate of 1 ton of seed per acre. Inevitably with granule treatment it takes time to get sufficient root development for the plant to pick up an adequate amount of menazon to produce control. In the trials, the aphid population had faded out by mid-July so we cannot say much about the relative persistence of granular and tuber treatments. The tuber treatment gave some protection right through to the middle of July.

Mr. Edgar added

The seed treatment is a placement technique getting the material exactly where it is wanted to give protection as soon as the seed germinates or the plant breaks through the soil, whereas with a granular treatment roots have to grow out before the toxicant is absorbed and the protection of the young plant may be delayed.

Mr. Newman

Carrots are one of the very few crops so far where we have obtained serious phytotoxicity with seed dressing. Foliar sprays might be more satisfactory

Mr. Edgar

I think Mr. Newman exaggerates phytotoxicity on carrots. It has occurred but I think we may be able to overcome this.

Q. Mr. Worthing

Can Mr. Elings give us any information of structure activity relationships in compounds of the WP 155 series? For instance, do small changes in structure completely remove the activity, or does the activity slowly tail-off with homologous compounds? How critical is the given structure?

A. Mr. H. Elings

We found quite a similar type of activity in rather a great number of derivatives of WP 155. So there are gradual changes in activity among members of the series and WP 155 is not to be considered an exception in it. WP 155 has been chosen also on other grounds as its excellent fungicidal properties.

Q. Mr. E. Robert

What is the mammalian or human toxicity of tin compounds?

A. Ir. A.J. Pieters

There are several data on the toxicity of triphenyl tin compounds, some of which you will find in the paper. In practice the toxicity of triphenyl tinhydroxide will for several applications give no difficulties. For instance it has been established that in potatoes no residues can be found in the tubers. Some data on the subject of chronic toxicity of the triphenyl tin compounds are known from literature. A Belgian publication named "Répertoire Toxicologique des Pesticides" of Simone Dormal and Georges Thomas (1960) states that 5 p.p.m. of triphenyl tinacetate in the food during 105 days caused no adverse effects to rats.

We can assume on physiological reasons that data on triphenyl tinacetate are applicable to triphenyl tinhydroxide also.

Q. Mr. I.F. Storey

During the past three years, we have carried out trials in Lincolnshire using tri-phenyltin acetate for the control of potato blight and have obtained very effective control both of the disease on the foliage and in the tuber. There has been no indication of any adverse effect on yield in spite of slight injury to the leaf from time to time. In the trials, we have obtained on average a yield increase of $1\frac{1}{2}$ tons per acre whereas with copper oxychloride the increase was of the order of 8 - 10 cwt per acre.

The tin compound has been the only one used which seems to have a direct effect on tuber blight. Is there any indication from the work in Holland how this effect has been obtained? Has there been a direct effect by protection of the tuber in the soil or is this the result of a toxic effect on the spores whilst present on the leaf?

A. Ir. A.J. Pieters

I regret not to be able to explain the mode of action of triphenyltinhydroxide on tuber blight. As far as we know no explanation of this phenomenon is available up till now. As far as herbicidal effects are concerned I do not think that we have seen such a thing in our trials; perhaps Mr. Elings could add to this being the man that has done the field trials.

Mr. Elings added

We sometimes see a slight scorching effect on some weeds, but they are by no means killed.

Q. Professor A.E. Muskett

Has Mr. Elings compared the effectiveness of WP 155 for the control of rose mildew with that for washing soda (2 oz per gallon) and milk?

A. Mr. Elings

The answer is no.

Q. Mr. A Casella

Could Mr. Higgons give information about toxicity of HOE. 2784?

A. Mr. D.J. Higgons

HOE 2784 is only a moderately toxic compound with an oral LD 50 to rat of 130 mg./Kg. It has however, an extremely low toxicity by dermal absorption, and should therefore, be a relatively safe compound to use in practice.

Q. Mr. Peter G. Burgess

Has Mr. Elings tried WP 155 against rose mildews in glasshouses, especially with the rose variety Baccarat?

A. Mr. Elings

Yes, we have. The activity against mildew in roses in glasshouses is as good as against mildew on outdoor roses. Baccarat was included in many tests; we did not observe any phytotoxicity on this variety.

Q. Dr. Ripper

Has Dr. Elings tried to control Erisyphe graminis with WP 155 and has it a curative effect in severe outbreaks? Is it phloem translocated? Is it compatible with the standard plant hormone weedkillers?

A. Mr. Elings

Quite a number of the laboratory experiments were done with powdery mildew on barley, which is one of our standard test plants for powdery mildew screening. We made on field-test against Erisyphe on barley and the effect of WP 155 was good. So it is perhaps possible you could use WP 155 on cereals if you wanted to, but I am not sure it would not be too expensive for these crops. We did not yet look further into this matter and we do therefore not know if the curative effect would be sufficient in severe outbreaks. Probably WP 155 formulations are compatible physically and chemically with 2,4D and MCPA-formulations. We do not yet know, how exactly WP 155 translocation takes place.

Q. Mr. H.G. Haigh

1. Have any comparative field trials of the organic tin compounds against Bordeau Mixture been carried out, similar to those described for copper oxychloride?

2. How does the cost of treatment with tin compounds compare with that for copper compounds, in view of the much higher price of tin?

3. In the tables in the paper, 50% presumably refers to the metal content of copper oxychloride. Does 20% refer to the metal content of the tin compound or does it refer to the % active ingredients?

A. Ir. A.J. Pieters

We have not used Bordeaux Mixture in our trials. Regarding the cost of the triphenyltinhydroxide, you have to realise that the concentration of the metal is very low as compared with copper. 50% refers to the metallic copper but 20% refers to the active compound viz triphenyltinhydroxide,

which contains about 30% metallic tin. Regarding the price I think that it is necessary and it certainly seems possible to sell triphenyltinhydroxide at prices that will only slightly be higher than the copper products and I think in practice these two products will be used.

NEW DEVELOPMENTS, INCLUDING NEW COMPOUNDS, NOVEL FORMULATIONS, OR NEW APPLICATION METHODS (cont.)

Chairman: A. E. Muskett
Queen's University of Belfast

ACARICIDE GROUP

THIOQUINOX* - A NEW SPECIFIC ACARICIDE OF THE QUINOXALINE GROUP

by K. M. Powell and W. Linke
Baywood Chemicals Ltd.

Summary

Thioquinox is a new acaricide based on quinoxaline-2,3-diyl-trithiocarbonate. It has a low mammalian toxicity and a specific action which is primarily directed against Tetranychidae. In trials carried out here and abroad in 1959/61 applications of 1 - 2 lb per acre of thioquinox 50% wettable powder have given consistently good results against M. ulmi, including organophosphorus resistant strains. Thioquinox was also effective against powdery mildew on apples but when used more than six times at 0.1% has shown phytotoxicity. No adverse effect has been noticed following up to 3 post-blossom applications of thioquinox at concentrations up to 0.1%.

In laboratory and field trials, thioquinox proved to be effective against Glasshouse Red Spider mite (T. telarius) including organophosphorus resistant strains.

Introduction

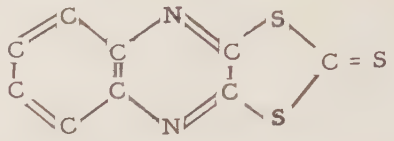
Since Unterstenhöfer (1960a) reported its acaricidal properties, we have carried out, since 1959, a number of field trials with thioquinox in this country to assess its efficiency against Fruit Tree Red Spider mite (M. ulmi) under British conditions and to obtain information on phytotoxicity and residues. A small scale trial was also made against powdery mildew (Podosphaera leucotricha) on apples. In this paper we give the results of these trials, at the same time quoting results of trials work done on the Continent and in South Africa, made available to us through the Biological Institute of Farbenfabriken Bayer A.G., Leverkusen.

Chemical, physical and toxicological properties of thioquinox

Reference to the chemical, physical and toxicological properties of

* Proposed common name for quinoxaline-2,3-diyl-trithiocarbonate

thioquinox is made by G. Unterstenhöfer (1960a) and K. Sasse (1960. Chemically, it is quinoxaline-2,3-diyl trithiocarbonate, a brownish odourless powder, insoluble in water and stable at temperatures up to 200° C. The chemical structure is:



Thioquinox was available as a 50% wettable powder formulation.

The toxicity of thioquinox was assessed by O. Klimmer (1960), the acute oral LD50 to rats being 3,400 mg/kg (± 447.1 mg/kg). The acute intraperitoneal LD50 on rats is given as 231.5 mg/kg. Doses up to 3000 mg/kg applied to the skin of rats caused no symptoms. According to A. Worden (1961) the oral LD50 to pheasants is approximately 2500 mg/kg.

Biological properties of thioquinox

Thioquinox is mainly an acaricide although it shows a slight aphicidal action under laboratory conditions. It has no effect on honey bees (Apis mellifera) and is classified as being harmless to bees by the German Federal Biological Institute. Ichneumonidae, Braconidae and Coccinelidae were found to be resistant to thioquinox (Unterstenhöfer, 1960). Reports from the U.S.A. suggest some effect on Psylla pyricola. The fungicidal action of thioquinox against powdery mildew on apples was noted by Sasse et al. (1960), and the Federal Biological Institute at Vienna has approved its use for the control of powdery mildew on grapes.

The effect of thioquinox on Tetranychidae is more pronounced than its effect on Tarsonemidae and Eriophyidae. In laboratory experiments Unterstenhöfer (1960b) found M. ulmi more susceptible to thioquinox than T. telarius, the lowest efficient concentrations being 0.005% and 0.008% respectively. He also established that thioquinox kills the summer eggs, the larval and nymphal stages as well as adults of M. ulmi and T. telarius; winter eggs of M. ulmi are affected to a lesser degree.

Thioquinox shows a considerable persistance, especially against larval stages which seem to be more susceptible than other stages. Its effect on Tetranychidae is slow and often a 100% kill of active stages can only be observed 5 - 7 days following application.

Laboratory work indicates that thioquinox is effective as a contact and stomach poison but nothing is known about its primary mode of action, i.e., which specific system if affects.

Field trials work against M. ulmi - 1959/61

In 1959 thioquinox and azinphos-methyl were included in a replicated trial at Rowhill Experimental Farm, Kent. Both materials were applied high volume on the variety Cox's Orange Pippin, James Grieve and Worcester Pearmain on the 28th May, 22nd June and 22nd July. The results expressed as mites per 30 leaves are given in Table 1.

Table 1. RED SPIDER TRIAL, ROWHILL FARM, 1959
Results (mites per 30 leaves) Trial 3 x replicated

Date of counts	Thioquinox (50% w.p.) 0.1%	Azinphos-methyl 0.2%	Control
4th June	1.3	0.6	241
18th June	3.6	7	301
2nd July	-	-	172
16th July	3.0	0.3	344
30th July	-	0.3	464
20th August	13	6	1,135
4th September	50	10	1,489

A similar trial was carried out at Rowhill in 1960 giving two applications instead of three. These were applied on the 2nd/3rd June and 26th/27th July. The results are given in Table 2.

Table 2. RED SPIDER TRIAL, ROWHILL FARM, 1960
Results (mites per 40 leaves) Trial 4 x replicated

Date of counts	Azinphos-ethyl		Thioquinox (50% w.p.)		Control
	0.05%	0.1%	0.05%	0.1%	
1st June	336	204	454	297	275
9th June	3	3	2	8	170
23rd June	4	1	10	3	320
7th July	9	5	12	5	568
21st July	9	5	31	22	298
4th August	2	3	5	2	678
25th August	5	5	13	9	837

A transformation of these results into percentage control (Henderson and Till) shows that the two thioquinox sprays resulted throughout the season in a degree of control which was never below 91.5% when applied at 0.1%, and 86.1% when applied at 0.5% of the formulated product.

Owing to a very slight infestation of M. ulmi on apples at Rowhill in 1961 it was decided to compare the effect of one application of thioquinox

and azinphos-methyl on Myrobalan plums which were heavily infested. A high volume application was made on 11th July. The results expressed as percentage control (Henderson and Till) are given in Table 3.

Table 3. RED SPIDER TRIAL, ROWHILL, 1961 ON MYROBALAN PLUMS
Results as percentage control (Henderson & Till)

Treatment	Date of assessment		
	13th July	24th July	1st August
Azinphos-methyl (20% emulsion) 0.2%	91	69	85
Thioquinox (50% w.p.) 0.075%	80	99	80

The three trials described were carried out on Fruit Tree Red Spider mites known to be susceptible to organophosphorus compounds. To obtain information on the effect of thioquinox against resistant strains of Fruit Tree Red Spider mites, a field trial was laid down in 1961 in an orchard in Essex, where previously organophosphorus compounds had given poor control. Here thioquinox was applied once on 25th May at a rate of 1 and $1\frac{1}{2}$ lb of a 50% wettable powder per acre in 250 gallons of water on the variety Cox's Orange Pippin. The results in Table 4 are expressed as the number of mites per 105 leaves.

Table 4

Date	Thioquinox (50% w.p.)	
	1 lb/acre	$1\frac{1}{2}$ lb/acre
Pre-spraying count		
25th May	3199	3390
Post-spraying counts		
1st June	16	21
8th June	6	2
19th June	5	5
3rd July	6	17
17th July	123	33
31st July	56	134

Further information of the effect of thioquinox on organophosphorus resistant Fruit Tree Red Spider mite is available from trials carried out by the Biological Institute of Farbenfabriken Bayer A.G. in the Rhineland in 1960. Table 5 gives the results (average of 7 trials) as percentage control at several intervals following one treatment with thioquinox, azinphos-methyl and demeton-S-methyl.

Table 5. TRIALS AGAINST OP-RESISTANT RED SPIDER MITE (M. ULMI) IN THE RHINELAND, 1960

Results expressed as percentage control (mean of 7 trials)

Treatment (Beginning of June)	Concentr. (a.i.)	Number of days after treatment 2-5	6-10	11-20	>20
Thioquinox	0.05%	100	94	96	95
Thioquinox	0.025%	84	97	93	95
Thioquinox	0.0125%	85	89	82	84
Azinphos-ethyl	0.04%	79	94	96	91
Demeton-S-methyl	0.025%	74	82	49	45

Effect of thioquinox on T. telarius (L.)

The results of two trials were made available to us by the Biological Institute of Farbenfabriken Bayer A.G. Table 6 gives the results of a laboratory trial against T. telarius on beans. The figures refer to the percentage control at different intervals after application following artificial reinfestation.

Table 6. LABORATORY TESTS AGAINST T. TELARIUS ON BEANS

	Percentage kill after days 1	3	10	17
Thioquinox 0.0125%	15	50	100	100
Thioquinox 0.025%	25	60	100	100
Thioquinox 0.05%	75	100	100	100

The effect of thioquinox, demeton-methyl and azinphos-methyl against organophosphorus resistant T. telarius has been assessed in a replicated field trial carried out in 1959 by the Technical Staff of Agro-Chem in South Africa. The results expressed as number of active stages per 120 leaves are given in Table 7.

Table 7. TRIAL AGAINST OP-RESISTANT RED SPIDER MITE (T. telarius) on apples (Var. "Cape Province") South Africa, 1959 (6 x replicated) Results (active stages/120 leaves.

Treatment	Pre-treatment	Post-treatment		
	23.3.59	28.3.59	6.4.59	16.4.59
Demeton-methyl (50% emulsion) 0.1%	1,814	428	575	650
Azinphos-methyl (25% w.p.) 0.15%	2,372	444	258	197
Thioquinox (50% w.p.) 0.1%	3,353	149	16	14
Control	2,703	2,244	1,180	812

W.H Read stated (personal communication) that thioquinox applied at 0.1% has given good results against organophosphorus resistant Glasshouse Red Spider mite in this country.

Effect of thioquinox against Powdery Mildew on apples

Thioquinox was included in a replicated trial at Rowhill Farm in 1960 on the variety Cox's Orange Pippin. Six high volume applications were given using a rate of 0.1% of the formulated product. Counts of secondary infections were made in August, 1960, the leaves being graded according to the severity of infection from 0 - 5. The figures in Table 8, expressing the results, were obtained by multiplying the number of leaves in each category by the category value and summing. Colloidal sulphur 40% is included as a comparison.

Table 8. MILDEW TRIAL, ROWHILL, 1960 Secondary infection counts August, 1960 Results (number of leaves in category (0-5) x category value)

Treatment	Block I	Block II	Block III	Mean
40% Coll. sulphur (0.5%)	24	37	27	29
Thioquinox (50% w.p.) (0.1%)	29	40	30	33
Control	65	94	82	80

Spraying Dates: 28.4 / 11.5 / 25.5 / 10.6 / 21.6 / 20.7

This assessment was followed up by flower truss counts in spring 1961, the results of which are given in Table 9 as percentage of mildewed clusters.

Table 9. MILDEW TRIAL, ROWHILL, 1960
Flower Truss Counts, Spring, 1961
Results (percentage mildewed clusters)

Treatment	Block I	Block II	Block III	Mean
40% Coll. sulphur (0.5%)	7.0	1.8	1.4	3.4
Thioquinox (50% w.p.) (0.1%)	9.8	4.8	6.7	7.1
Control	15.3	16.4	13.4	15.0

It must be pointed out that, in this trial, the plots treated with colloidal sulphur had already received a series of sulphur sprays in 1959 when this mildew trial was started, whereas thioquinox was used on previously unsprayed trees. Hence the initial mildew infection in the thioquinox plots was higher in the spring of 1960.

In an unreplicated field trial against powdery mildew on apples carried out in Essex in 1961, plot 1 received 6 sulphur and 3 thiram sprays during the season. In plot 2 thioquinox was used twice post-blossom, primarily for Red Spider control and on these occasions the sulphur spray was omitted and replaced by thiram. 200 leaves per plot were sampled in August and graded 0 - 5 in order to assess the incidence and severity of secondary mildew infections. By multiplying the category value by the number of leaves in each category and obtaining a total, an index figure was arrived at which was 336 in plot 1 and 287 in plot 2, indicating that the applications of thioquinox have helped to maintain mildew control.

In Holland (Wäckers, 1960) replicated trials on the variety Jonathan were carried out to assess the effect of thioquinox on secondary infections of Powdery Mildew when used in combination with thiram and another organic fungicide referred to as "Product X". 10 spray applications were given from the 21st April to the 13th July. An assessment of mildew was made on 500 leaves per treatment and the results, expressed as the percentage infection, are given in Table 10.

Table 10.

Thioquinox 0.05% + Product X	11.2%
Thioquinox 0.05% + thiram	7.2%
Thioquinox 0.1% + Product X	11.2%
Thioquinox 0.1% + thiram	4.4%
Fungicide X	83%

Effect of thioquinox on plants

Observations on the effect of thioquinox on plants were made at Rowhill Farm from 1959 to 1961. Under the conditions of these trials up to 3 applications of thioquinox (50% wettable powder) at concentrations up to 0.1% did not lead to an increased russetting on apples of the varieties Cox's Orange Pippin, James Grieve, Laxton's Superb, Bramley's Seedling, Worcester Pearmain and Tydeman's Early Worcester, nor was there any phytotoxic effect on the leaves. Where thioquinox was applied six times in a season at a concentration of 0.1% on the variety Cox, a higher degree of russetting was noticed compared with the control and the plots treated with colloidal sulphur 40% at 0.5%. The leaves, in comparison to the control, were harsher, of a lighter colour and brittle in texture. Similar observations were made in Holland (Wackers, 1960) where 7 - 10 applications of thioquinox at concentrations of 0.1% caused leaf damage whilst 3 sprays at a concentration of 0.1% and 7 sprays at 0.05% had no adverse effect. In these trials a brown discolouration of petals was noticed when thioquinox was sprayed on apple trees in full blossom. In trials carried out in the Rhineland, the effect of thioquinox on 45 apple varieties was studied. 2 - 3 sprays high volume at rates between 1 and 2 lb of the 50% wettable powder per acre caused yellowing of the leaves on two varieties only, namely James Grieve and Berlepch whilst the other varieties were unaffected.

In two trials leaf damage on beans (Phaseolus vulgaris) was noticed following the application of thioquinox at 0.1%. Hops seem to be very sensitive to thioquinox and its use on this crop cannot be recommended. In the United States no damage was noticed on 150 varieties of roses following the application of thioquinox.

Thioquinox Residues

Residues on plant material can be assessed using the method described by Tietz, Osman, Frehse and Niessen (1960).

At the time of writing, the full results of this year's residue tests on apples and plums treated in this country are not available. On apples of the variety Golden Delicious, treated in South Africa with thioquinox at 0.1% a residue of 1.3 p.p.m. was found on the day of the spray treatment which decreased to 0.88 p.p.m. within a fortnight.

Discussion of the results

Thioquinox has, under trials conditions, given consistently good results against Fruit Tree Red Spider mite on apples and Myrobalan plums in this country and in trials on the Continent. A concentration of 0.1% of the 50% wettable powder formulation was slightly more effective than the concentration of 0.05% but the differences may not be significant. It is difficult to judge the true residual effect of acaricides from field trials but all our observations, as well as those from workers in other countries, indicate that thioquinox has a fairly long persistence, especially against the larval stages. In years not favourable to the development of Fruit

Tree Red Spider mite one spray application after completion of winter egg hatch would probably be sufficient to maintain good red spider control throughout the season. In "red spider years" a second application 3 - 4 weeks after the first application might become necessary. As thioquinox affects summer eggs as well as all active stages, the larvae being more susceptible than the adults, timing is thought to be less critical than with acaricides whose action is limited to certain development stages only. Trials evidence and observations from here and abroad show that thioquinox is equally effective against "normal" and "resistant" strains of M. ulmi. This property and the chemical structure of thioquinox indicates an entirely new mode of action. This development appears to be a step in the direction indicated by Collyer and Kirby (1959).

Thioquinox would seem to be particularly suitable for use in areas where resistance to acaricides in current use has already occurred and also where growers wish to adopt a "rotation" in the use of acaricides in order to prevent or delay the development of resistance. Its low mammalian toxicity, its harmlessness to bees and probably to other beneficial insects and its specific action are noteworthy advantages. More detailed studies on the effect of thioquinox on important predators in this country are, however, desirable.

There is evidence that where thioquinox is used against red spider in the apple spray programme, the addition of a specific mildew fungicide is not necessary, although further proof on this point should be obtained. Because of injury to apple trees of six or more applications of thioquinox at a concentration of 0.1%, a full mildew programme at this concentration must be ruled out. Further work using lower concentrations will, however, be carried out. Meanwhile we are satisfied that up to three post-blossom applications of thioquinox (50% wettable powder) at 0.1% will not cause any damage to apples or plums. On crops other than fruit there are indications that thioquinox could be useful against T. telarius, particularly organophosphorus resistant strains.

Acknowledgements

We gratefully acknowledge the help given to us by fruit growers in this country in making plots for trials work available. Thanks are also due to Mr. M. D. Austin who supervised part of the field trials work in 1961.

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2,4,5,4'-TETRACHLORO-DIPHENYL SULPHIDE, AN ACARICIDE WITH OVO-LARVICIDAL PROPERTIES*

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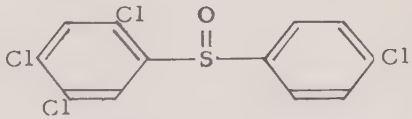
Introduction

In 1952 2,4,5,4'-tetrachloro-diphenyl sulphone (tetradifon) was shown to possess extremely high acaricidal properties (Meltzer, 1955). As field trials and practical experience have proved this substance useful for mite control (Flik, 1957), a large group of related compounds have been examined (Huisman, et al., 1958).

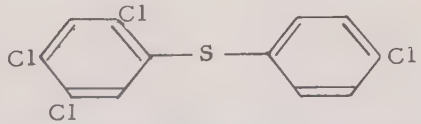
In this paper the acaricidal properties of the sulphide homologue of tetradifon are compared with tetradifon and its sulphoxide homologue.

2,4,5,4'-tetrachloro-diphenyl sulphone

2,4,5,4'-tetrachloro-diphenyl sulphoxide



2,4,5,4'-tetrachloro-diphenyl sulphide



To examine the direct ovicidal action, beanplants (Phaseolus vulgaris) bearing eggs of Tetranychus telarius** were dipped in emulsions or suspensions of the compounds concerned. Larvicidal action following the hatch of the surviving eggs completes the acaricidal action. To examine the residual action of the compounds, dipped beanplants were infested with female mites after the deposit had dried. A detailed description of the methods used was given by Meltzer (1955).

It appeared that leaf penetration contributes much to the acarcidal action in this type of compound, and experiments were carried out to examine this property. In these experiments the upperside only of the leaves

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* Communication no 63 of the Agrobiological Laboratory "Boekesteyn", 's-Graveland, The Netherlands.

** The species of the telarius complex used are T. urticae, T. hydrangeae and T. cinnabarinus.

was treated with an emulsion, applied by means of an Agla micro-syringe and distributed over the whole surface with a glass rod. By using eggs on the underside of the leaves only, laid before and after application of the substances, the results give an indication of the penetration, and even of the relative speed of penetration of the compounds concerned.

In conclusion experiments were made with the fruit tree red spider mite, and, in particular, the action on wintereggs was studied.

Initial and residual ovo-larvicidal activity

With respect to the initial activity it will be seen from Table 1, that the sulphone possesses the highest strictly ovicidal activity. The inferior contact action of the two other compounds, however, is largely compensated by larvicidal action. It is known, from other experiments, that the mortality of larvae and nymphs at the lower concentrations is maindly due to oral intoxication (Meltzer, 1957a). So it may be assumed, that this compensation is due to a relatively strong penetration of both the sulphoxide and the sulphide into the leaf.

Table 1. Initial ovo-larvicidal activity of 2,4,5,4'-tetrachloro-diphenyl sulphone, -sulphoxide, and -sulphide on Tetranychus telarius. Mean percentage mortality of at least six experiments. E = percentage egg mortality; T = total kill (eggs and larvae together).

Concentration in p.p.m.	Sulphone		Sulphoxide		Sulphide	
	E	T	E	T	E	T
30	98	100	90	100	60	100
10	95	100	63	98	52	98
3	74	98	48	96	23	88
1	32	90	15	60	15	60

The residual activities, i.e. the activity of the dry deposit on the leaf of the three compounds are similar (Table 2), though the strictly ovicidal activity of the sulphone is superior to both the other compounds. As has been shown in earlier work (Meltzer, 1957b) egg kill is mainly due to oral uptake of the deposit from the leaf by the female mites. In the experiments of Table 2, the period of three days between treatment and deposit of the lastly laid eggs enabled the compounds to penetrate and the ovicidal action is sharply increased with relation to the initial effect.

Table 2. Residual ovo-larvicidal activity of 2,4,5,4'-tetrachloro-diphenyl sulphone, -sulphoxide and -sulphide on Tetranychus telarius. Mean percentages mortality of at least six experiments.
E = percentage egg mortality; T = total kill.

Concentration in p.p.m.	Sulphone		Sulphoxide		Sulphide	
	E	T	E	T	E	T
30	100	100	93	100	88	98
10	100	100	89	97	83	99
3	92	100	85	98	71	96
1	24	69	55	62	32	66

The duration of the residual action of the three compounds, however, is different, as can be seen from Table 3. The sulphone- deposit shows the most lasting action; the sulphide the least, whereas the sulphoxide takes an intermediate position.

The higher initial egg killing activity of the sulphone, and particularly the longer residual action led to the choice of the sulphone (tetradifon) for spider mite control during summer.

Table 3. Residual action of miscible oil preparations of 2,4,5,4'-tetrachloro-diphenyl sulphone, -sulphoxide, and -sulphide. E = percentage egg mortality; T = percentage total mortality.

Infestation after	Conc. (p.p.m.)	Sulphone		Sulphoxide		Sulphide	
		E	T	E	T	E	T
	300			100	100	100	100
	100	100	100	100	100	99	100
0 days	30	100	100	97	100	100	100
	10	93	98	88	95	97	100
	3	91	97	31	83	95	99
	300			98	100	97	100
	100	93	99	87	99	85	99
7 days	30	86	99	74	97	79	98
	10	81	96	41	55	67	77
	3	39	83	9	12	5	6
	300			77	100	82	99
	100	97	99	81	100	80	95
14 days	30	95	99	30	73	34	58
	10	83	97	28	43	22	26
	3	49	69	9	11	6	9
	300					61	78
	100	80	100	41	83	43	46
21 days	30	78	94	11	31	23	27
	10	63	97	3	3	0	0
	3	24	55	5	5	0	0

Leaf Penetration

The different leaf penetrating properties of the three compounds concerned have been reported earlier (Meltzer and Dietvorst, 1957). The results may be briefly summarized here, because leaf penetration and duration of action, and leaf penetration and action on winter eggs seem to be correlated.

The treatment of the upperside of bean leaves of about 30 cm^2 with 0.1 ml of an emulsion of the compounds concerned will cause mortality of eggs and larvae on the underside of the leaves. In the experiments recorded in Table 4, the sulphide gave the best results.

On leaves treated after the mite eggs have been laid, only the sulphide is capable of killing a certain number of them. The eggs that aged three days, and which were just about to hatch at the time of application, were not killed. So this substance is not only able to penetrate into the leaf, but also into the eggs. On leaves treated before the eggs are laid on the opposite side, all compounds exerted ovicidal action, though again the sulphide appears to be superior in relation to leaf penetration properties.

Table 4. Leaf penetration of 2,4,5,4'-tetrachloro-diphenyl sulphone, -sulphoxide, and -sulphide. Only the upperside of the leaf is treated. Eggs of Tetranychus telarius at the underside only, 0 - 3 days old.
E = percentage egg mortality; T = percentage total mortality.

Compound	Dosage ug/leaf	Eggs laid before treatment		Eggs laid after treatment	
		E	T	E	T
Sulphone	100	1	100	79	100
	10	1	100	41	100
	1	2	30	22	53
Sulphoxide	100	9	94	95	100
	10	2	100	56	99
	1	3	23	9	57
Sulphide	100	68	100	100	100
	10	3	100	95	100
	1	1	82	26	98

Activity on Metatetranychus ulmi Koch

In earlier work the summer eggs of the fruit tree red spider mite was found more susceptible to the sulphone (tetradifon) than were the eggs of Tetranychus telarius (Meltzer, 1957c). It appeared, that the summer eggs of Metatetranychus are also more susceptible to the sulphide than the eggs of Tetranychus.

The differing leaf penetration properties of the three compounds led us to compare their effects on the wintereggs of Metatetranychus ulmi (Meltzer, in the press). The results demonstrate that the sulphide, which shows the best leaf penetration also possesses the highest activity on the wintereggs (Table 5).

Table 5. Activity of 2,4,5,4'-tetrachloro-diphenyl sulphone, -sulphoxide, and -sulphide, as compared with fenson on winter eggs of Metatetranychus ulmi. Mean percentage reduction of hatch after dipping egg-bearing apple twigs in emulsions (three experiments).

Concentration in p.p.m.	Sulphone	Sulphoxide	Sulphide	Fenson
500	7	7	100	71
250	10	14	90	61
125	5	0	87	29

In later experiments with emulsifiable concentrates and wettable powders of the sulphide at lower concentrations, no difference in activity was found between the two types of formulation. (Table 6).

Table 6. Percentage reduction of hatch of winter eggs of Metatetranychus ulmi, after treatment with emulsifiable concentrate and wettable powder of the sulphide.

Concentrations act.mat.(p.p.m.)	Emulsifiable concentrate (6 expts.)	Wettable powder (3 expts.)
300	100	100
100	97	99
30	96	97

Field experiments with the sulphide

Field experiments on wintereggs of the fruit tree red spider were carried out at two application dates, viz. at the green tip stage, and at the time that hatching of wintereggs is beginning. The first date is the time for the application of the DNC/mineral oil winter wash; the latter the optimal time for application of fenson.

Table 7 summarizes some results obtained with preparations of the sulphide as compared with the standard DNC/mineral oil and fenson for the two dates of application.

Table 7. Mite counts of field experiments on winter eggs of Metatetranychus ulmi. Mean counts of samples of four replicates of 100 leaves each.
Time of application: Golden Delicious I March 23; II April 24.
Cox's Orange Pippin I March 20; II April 29.

Material and concentration	Time of spray	Mite Counts per 100 leaves Golden Delicious	Cox's Orange Pippin	
		May 19	May 14	May 25
Sulphide, 20% e.c., 0.2%	I	0.3	0.1	0.2
	II	0.0	0.1	0.05
Sulphide, 20% w.p., 0.2%	I	0.5	0.2	0.4
	II	0.05	0.2	0.3
DNC/mineral oil 6.0%	I	0.1	0.0	0.1
Fenson, 50% w.p., 0.1%	II	0.1	0.05	0.1
No treatment		4.7	0.9	4.2

Trials of concentrations below 0.2% are still going on. For the time being the concentration of 0.2% is recommended if hatching of the winter eggs occurs before or at the beginning of blooming; later 0.1% will suffice. The satisfactory results with 0.1% with later applications can be explained by the better action at increased temperature and the larger leaf surface present results in a greater amount of active material absorbed by the leaves, and as a consequence a high larvicidal effect.

Earlier it was found, that tetradifon effectively killed the first generation of the fruit tree red spider when applied after about 75% hatch of the winter eggs, and this is now readily understood, for this sulphone is not an effective ovicide but kills the larvae on the leaves. With the sulphide, however, the results are equal, whether there is a hatch of 20% or 60% or more at the time of application.

Many large scale field trials were carried out in Belgium and in the Netherlands during 1960 and 1961 on as many apple varieties as possible. The results of these trials confirmed those of the previous experiments, and, in most cases the mote control appeared to be so efficient that no further control measures were necessary during the whole season. In none of these trials have phytotoxic symptoms been observed.

The mammalian toxicity of the sulphide is low; the LD 50 for rats and mice, both oral and intraperitoneal exceeds 14700 mg/kg. Just as is the case with tetradifon, the sulphide appears to be a highly selective acaricide, safe for insect predators, bees, animals and human beings.

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INVESTIGATIONS OF A NEW SERIES OF NITROGENOUS ORGANOPHOSPHORUS COMPOUNDS. CHARACTERISTICS AND PROPERTIES.

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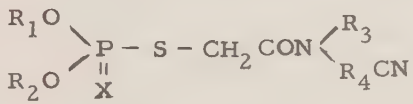
Summary

The series of nitrogenous organophosphorus compounds in question has the general formula:



where X stands for O or S, R_1 and R_2 stand for identical or different alkyl radicals, R_3 is H or an alkyl radical, and R_4 is an aliphatic hydrocarbon chain.

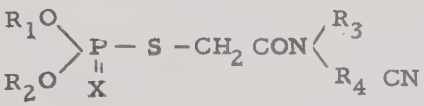
The insecticide and acaricide actions of these compounds were examined in correlation with their structure and properties.

From the laboratory and field tests described the compound $(C_2H_5O)_2$ PO.S.CH_2.CONH.$C(CH_3)_2$CN was chosen for further tests.

1. Introduction

Research has proceeded for some time in our laboratories on organophosphorus compounds with insecticidal and acaricidal action (Fusco et al., 1959, 1960; Losco and Peri, 1959; Rossi, 1959) and has led to a new class of compounds (B. P. appl. 39743/60; IV. P. 625074; IV. P. appl. 17266/60). This class, and one of its members in particular, has been found to possess interesting properties in its action on crop pests.

These compounds have the general formula:



where: X is oxygen or sulphur; R_1 and R_2 are CH_3 and CH_3, or CH_3 and C_2H_5, or C_2H_5 and C_2H_5; R_3 is H or CH_3 or C_2H_5 and R_4 is CH_2 or $(CH_2)_2$ or $C(CH_3)_2$.

In the present paper we shall only consider those combinations of the above substituents on which screening values have been completely determined.

The compounds are of an oily nature, though in some cases, crystallisation occurs after prolonged storage.

These compounds conform to the rule (Rossi, 1959) that their solubility in water depends on the presence of the P=O linkage, for those with the P=S linkage are of low water solubility.

The insecticidal and acaricidal activity of the compounds of this class was determined by standard laboratory methods; in some cases field tests were also carried out.

In the laboratory, screening methods, which were standardised and therefore reproducable, were systematically applied to similarly formulated compounds; hence the results permitted the determination of the influence exerted by the various substituent groups on the insecticidal and acaricidal activity of the compounds.

In the field tests, commercial-type formulations of the chosen compounds were used and operations were carried out according to normal practice. Parameters and results of the experiments were recorded.

The toxicological findings of the screening, carried out on the usual laboratory animals, indicated fairly high acute toxicity values. These were, however, equal to or lower than those of many other substances already in commercial use. The values found for cumulative toxicity and for medium-term chronic toxicity of one of the field-tested compounds, viz. No.21 (code number 1568), would indicate a rather rapid metabolism. The latter, it is presumed, does not permit accumulation in the organism and thus reduces the hazard. It is not yet possible to give more precise details, for the chronic toxicity tests are still in progress.

2. Evaluation by laboratory methods of insecticidal and acaricidal action.

A) Methods

The methods of screening used will now be described schematically. A sufficient number of repetitions was made for each test and the results obtained were then used for finding the LD50 and the LD 95 values.

I. Contact Aphicidal activity with Aphis fabae:

Small, 10cm-high broad bean plants (Fava di Siviglia variety), previously infested with parthenogenetic apterous A. fabae females, were dipped for 5" in aqueous dispersions of graded doses. The plants were then kept at a temperature at 26 $\pm$ 1° C and a relative humidity of 60 $\pm$ 5% and illuminated for 18 hours per day with fluorescent lamps (2,000 to 2,500 lux at leaf level). Insect mortality was recorded twenty-four hours after treatment.

II. Systemic aphicidal action through the roots on A. fabae:

The roots of the broad bean plants (variety and size as above) were dipped in an aqueous dispersion of the compound, and the leaves were infested with A. fabae females. The plants were then kept seven days under the environmental conditions of the contact test I and examined every day, the final mortality being recorded after seven days.

III. Contact acaricidal activity on Tetranychus telarius:

Bean plants (Borlotto lingua di fuoco "Flame-tongued" variety) with two cotyledonary leaves only were infested with T. telarius adults and treated with aqueous dispersions of the compounds under examination. Mortality was recorded after 24 hours, the plants meanwhile being held in the controlled environment of Test I.

IV. Activity on eggs of Tetranychus telarius:

Bean plants (variety and stage of development as under III.) on which female T. telarius eggs had been deposited during the previous 24 hours, were treated with aqueous dispersions of the compounds as in Test I and kept in the controlled environment for six days; the percentage of unhatched eggs was then determined.

V. Stomach poison action on Leptinotarsa decemlineata:

Larvae ten days old and weighing 70mg each were introduced, one per cell, into special cellular containers designed to avoid any possible contact action. Each larva had access to an area of 2cm of young potato leaves (Agnes variety), previously treated with 0.01 ml of aqueous dispersions of the compound under examination at graded concentrations. Tests were carried out in the controlled environment of Test I and mortality was recorded after twenty hours.

B) Results

Table 1 lists the compounds examined and Table 2 gives, for each compound in the same order as in Table 1, the LD50s found by the five methods described. The LD 95s were also determined, but are not given as they follow the same order as the LD50s.

It is evident from Table 2 that certain of the compounds show interesting properties both in intensity and in range of action. Compounds M1568, M1567 and M1506 were selected for field-testing.

It was also considered advisable to subject compounds M1553 and M1571 to field tests in case the field conditions, where factors differ from those in the laboratory, have an influence on the order of toxicity.

The acute LD50s of the field-tested compounds for white mice, both oral and by injection, are given in Table 3.

D) Field experiments

The field tests were carried out during the years 1960 and 1961. The pests examined were: Aphids: Hyalopterus pruni, Yezabura malifoliae, Aphis pomi; Acarids: Metatetranychus ulmi.

The compounds chosen were all used in an emulsifiable form containing 20% active ingredient in the case of Nos. 1506, 1553, 1568 and 1571, and 10% in the case of No. 1567.

In all the experiments OO-diethyl S-(N-isopropylcarbamoylmethyl)

phosphorothiolothionate (FAC 20) was used as a reference, also in emulsifiable from containing 20% of active substance.

a) Tests on Hyalopterus pruni:

The specific purpose of the first test was to determine the action of the compounds on the aphid under field conditions, when the spray is applied mostly to the upper surface of the leaves of a heavily infested peach tree.

Spraying was carried out on 9th June, 1960, at Signa (Tuscany), using a hand-pump of 9 atmospheres pressure; the results of the test are given in Table 4.

In the second test, spraying was carried out on 23rd June, 1960, at San Benedetto Po (Lombardy) on a fine day, using a knapsack sprayer. Compounds M1568 and M1506 at a concentration of 600 p.p.m. and "F.A.C.20" at 300 p.p.m. of active substance, gave complete mortality of the aphids 24 hours after treatment, whereas the infestation of the control plant was unchanged.

In the third test, spraying was carried out on 26th June, 1960, at Cesena (Emilia), on a fine day with the temperature at 24 to 27° C, using a motor-pump at 30 atmospheres. Three peach-trees, distributed uniformly in two rows and all infested heavily were used. A complete control was obtained with M1568, M1506 and "F.A.C.20" at 250 p.p.m.

b) Test on Yezabura malifoliae

Treatment was carried out on 27th May, 1960, at Migliaro (Emilia), with a motor-pump at 20 atmospheres pressure, on apple-trees (Stark Delicious variety) ten years old and heavily infested. The results of the test are given in Table 5.

c) Tests on Aphis pomi:

The first group of tests consisted of four carried out in the months of June and July, 1960, in the following localities, all in Lombardy; Guidizzolo, Bondeno di Gonzaga, San Benedetto Po, and Pecorara di Pietra de' Giorgi.

The compounds were applied with a hand-sprayer and used at the following concentrations (p.p.m.):-

M1506:	600	-	500	-	300	-	250	-	-
M1553:	-	-	500	-	-	-	250	-	-
M1567:	-	-	-	-	-	-	250	-	125
M1568:	600	-	500	-	300	-	250	-	125
M1571:	-	-	500	-	-	-	250	-	-
"F.A.C.20"			:	-	300	-	250	-	-

The mortality of the aphids, determined 48 hours after treatment, was complete with all compounds.

Only one compound, M1568, was examined in the second group which consisted of five tests, carried out in Emilia and Lombardy in the months of May and June, 1961.

Complete mortality determined 48 hours after treatment was obtained with both No. 1568 and "F.A.C.20" at the 300 p.p.m. and the 200 p.p.m. levels. It appeared, as shown in Table 6 that No. 1568 was the faster acting of the two compounds.

d) Tests on Metatetranychus ulmi:

Many tests were carried out in 1960, in various regions of Italy and a few of the more important will be described here, including those carried out at San Bartolomeo in Bosco (Emilia) where, it was found that the mites were showing resistance to certain organophosphates. Motor-sprayers at pressures of 20 to 30 atmospheres were used for the application of the aqueous dispersions of test compounds. The results are given in Table 7 and 8.

The results of a third test, carried out on 1st July, 1960, at San Bartolomeo in Bosco (Emilia) are given in Table 9. Each plot consisted of three ten-year-old apple-trees (Stark Delicious variety). Infestation was extremely high. Before the tests, resistance to certain organophosphates had been noted.

Results of a fourth test, carried out on the 28th and 29th July, 1960, at San Bartolomeo in Bosco (Emilia) are given in Table 10.

A fifth test was carried out on 13th August, 1960, at Pegognaga (Lombardy) on rows of young apple-trees, Stark Delicious variety, of medium infestation. The determination of the infestation was made sixteen days after treatment by counting the live mites on forty leaves chosen from each tree of each treatment. The results are given in Table 11.

A sixth test was carried out on 5th July, 1961, at Guistella (Lombardy) each plot of two heavily infected apple trees. Both compounds M1567 and 1568, as well as the standard "F.A.C.20", at 300 p.p.m. eliminated the mites within two days.

It may be deduced from these results that, in general, all of the compounds under examination in the field are effective against Metatetranychus ulmi, though their efficiency was lower against organophosphate resistant mites except in the case of compound M1568.

Discussion

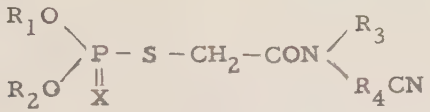
Comparisons of the structures of the compounds (Table 1) with the results obtained in the laboratory (Table 2) reveals that the introduction of certain substituents is frequently accompanied by an improvement or alternatively a deterioration, in their toxicity. The substituents R_1 and $R_2 = CH_3CH_3$; $R_3 = C_2H_5$; $R_4 = CH_2$ are more frequent in the compounds whose LD50 is higher, whereas the substituents R_1 and $R_2 = C_2H_5$ and C_2H_5; $R_3 = H$; $R_4 = C(CH_3)_2$ are more frequent in those compounds whose LD50 is lower. Compare, for example, compound M1563, which has the

highest LD50 (and thus the lowest level of action) in four of the five screenings, and, at the other end of the scale, M1568, whose LD50 is the lowest on A. fabae both by contact and systemically, and M1567 its sulphur analogue whose LD50 is the lowest on T. telarius, both adults and eggs.

The field tests carried out on the compounds under examination, although unfortunately cut down during 1961 for a number of reasons, have so far given good results against aphids and mites. In the case where the latter were resistant, a reduction in efficacy is apparent, but M1568, followed by M1567, proved on the whole to be the best of all the compounds examined, as in the screening tests.

In the field tests M1568 was also clearly superior to the reference substance "F. A. C. 20" whose practical value as an acaricide and aphicide is well known. In view of the results, M1568 is to be used on a wider scale during the coming season.

Table I. Compounds tested
General formula of the class:



Compound	Substituent Groups				
Code	R_1	R_2	R_3	R_4	X
M1557	CH_3	CH_3	H	CH_2	S
M1668	CH_3	C_2H_5	H	CH_2	S
M1556	C_2H_5	C_2H_5	H	CH_2	S
M1507	CH_3	CH_3	CH_3	CH_2	S
M1506	C_2H_5	C_2H_5	CH_3	CH_2	S
M1563	CH_3	CH_3	C_2H_5	CH_2	S
M1669	CH_3	C_2H_5	C_2H_5	CH_2	S
M1553	C_2H_5	C_2H_5	C_2H_5	CH_2	S
M1565	CH_3	CH_3	CH_3	$(CH_2)_2$	S
M1670	CH_3	C_2H_5	CH_3	$(CH_2)_2$	S
M1560	C_2H_5	C_2H_5	CH_3	$(CH_2)_2$	S
M1571	CH_3	CH_3	H	$C(CH_3)_2$	S
M1671	CH_3	C_2H_5	H	$C(CH_3)_2$	S
M1567	C_2H_5	C_2H_5	H	$C(CH_3)_2$	S
M1626	CH_3	CH_3	CH_3	$C(CH_3)_2$	S
M1625	C_2H_5	C_2H_5	CH_3	$C(CH_3)_2$	S
M1555	C_2H_5	C_2H_5	H	CH_2	O
M1505	C_2H_5	C_2H_5	CH_3	CH_2	O
M1554	C_2H_5	C_2H_5	C_2H_5	CH_2	O
M1564	C_2H_5	C_2H_5	CH_3	$(CH_2)_2$	O
M1568	C_2H_5	C_2H_5	H	$C(CH_3)_2$	O
M1624	C_2H_5	C_2H_5	CH_3	$C(CH_3)_2$	O

Table 2. LD50 of the compounds in laboratory tests

Compound Code	On A. fabae contact p.p.m.	On A. fabae systemic p.p.m.	On T. telarius contact p.p.m.	On T. telarius (eggs) p.p.m.	On L. decemlineata ingestion γ/insect
M1557	1,70	0,200	3,00	>1000	60
M1668	1,70	0,100	2,00	800	80
M1556	1,80	0,310	4,00	1000	32
M1507	0,70	0,125	1,70	>1000	7
M1506	0,70	0,125	1,00	400	5
M1563	6,00	0,600	120,00	>1000	>80
M1669	3,20	0,500	2,00	>1000	60
M1553	1,25	0,320	1,40	200	27
M1565	2,00	0,150	3,50	>1000	>80
M1670	1,50	0,080	0,30	500	32
M1560	1,25	0,100	2,00	>1000	15
M1571	2,00	0,300	0,50	200	>80
M1671	0,70	0,120	0,40	46	40
M1567	0,80	0,160	0,20	44	60
M1626	1,40	0,160	1,25	>1000	46
M1625	1,30	0,130	1,20	60	17
M1555	1,20	0,100	3,00	>1000	>80
M1505	1,20	0,150	2,50	>1000	3
M1554	1,60	0,200	2,50	>1000	16
M1564	1,60	0,060	1,00	>1000	>80
M1568	0,50	0,050	0,40	64	8
M1624	1,25	0,100	4,00	>1000	15
Dimethoate	0,30	0,050	1,80	-	30
"F.A.C.20"	0,60	0,20	1,00	220	37
Parathion	1,30	-	3,00	-	1
Chlorfenson	-	-	-	300	-

Table 3. Acute LD50 on white rat of compounds used in field tests

Compound	Acute oral LD50 mg/kg	Acute intravenous LD50 mg/kg
M1568	21	13
M1567	25	17
M1506	4	5
M1553	11	12
M1571	31	28
Parathion	8,2	5,7

Table 4. Field test on Hyalopterus pruni

Compound	Quantity p.p.m. of a.s.	Diminution of infestation after:		
		6 hours	24 hours	48 hours
M1568	300	50% nearly	nearly total	total
M1506	300	50% nearly	nearly total	total
"F.A.C.20"	300	50% nearly	nearly total	total

Table 5. Field tests on Yezabura malifoliae

Compound	Quantity p.p.m. of a.s.	Observations 70 h. after treatment	
		No. of branches examined	No. of living aphids
M1568	600	16	0
M1506	600	45	0
M1553	500	50	0
M1567	250	50	0
M1571	500	41	0
"F.A.C.20"	300	46	0
Unsprayed	-	51	45 colonies

Table 6. Field tests on Aphis pomi

Compound	Quantity p.p.m. of a.s.	Diminution of infestation after:		
		6 hours	24 hours	48 hours
M1568	300	93%	100%	100%
"F.A.C.20"	300	78,3%	99,7%	100%

Table 7. First field test on Metatetranychus ulmi

Compound	Quantity p.p.m. of a.s.	Living adult females per/leaf after		
		2 days	10 days	16 days
M1506	300	0,01	0,3	0,8
M1553	300	0	0,2	0,1
M1568	300	0	0,07	0,02
"F.A.C.20"	300	0	0,16	0,3
Control	-	4,5	37	13,4

Sprayed 7th June 1960 each on three Stark Delicious apple trees at Cesena (Emilia). Adult females counted on 300 leaves per treatment.

Table 8. Second field test on Metatetranychus ulmi

Compound	Quantity p.p.m. of a.s.	Living adult females per/leaf after			
		2 days	9 days	15 days	23 days
M1506	300	0,30	0,16	5,6	2,6
M1553	300	0,07	0,02	3,0	0,7
M1567	150	0,13	0,19	3,3	1,5
M1568	300	0,02	0,01	0,3	0,03
M1571	300	0,15	0,17	2,5	0,6
"F.A.C.20"	300	0,18	0,21	2,3	1,1
Control	-	25	36	45	42

Sprayed 6th June 1960 on Stark Delicious apples at Cesena.

Table 9. Third field test on Metatetranychus ulmi

Compound	Quantity p.p.m. of a.s.	No. of living mites per/leaf after	
		6 days	18 days
M1506	500	27	13
	250	-	48
M1553	500	12,5	6
	250	60	30
M1567	250	13,5	11
	125	40	20
M1568	500	1,5	1,5
	250	9	9
M1571	500	27	43
	250	39	55
"F.A.C.20"	500	4,5	2
	250	4	8
Control		113	147

Table 10. Fourth field test on Metatetranychus ulmi

Compound	Quantity p.p.m. of a.s.	No. of living mites per/leaf after	
		8 days	21-22 days
M1506	600	9	3
M1553	500	4	0,5
M1567	250	2,5	1
	500	7	3
M1568	300	1,5	0,25
	500	0,3	0,15
M1571	500	7,5	7,5
"F.A.C.20"	300	11	8
	500	7,5	5

Table 11. Fifth test on Metatetranychus ulmi

Compound	Quantity p.p.m. of a.s.	No. of living mites per/leaf after 16 days
M1553	500	0,4
M1567	250	0,7
M1568	300	0,1
	500	0,02
"F.A.C.20"	300	0,7
	500	0,1
Unsprayed	-	19

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INVESTIGATION INTO THE BIOLOGICAL ACTIVITY OF A SERIES OF BUTYLENE POLYMERS

by Ian Greenfield
F. W. Berk & Co. Ltd.

Introduction

The use of synthetic pesticides in the field creates many problems, not the least of which are toxicity to the operator, toxic residues on edible crops, development of resistant strains of pests, and the destruction of natural predators.

In view of these factors, the use of "Indopol"* polybutenes as experimental acaricides and fungicides in trials made by R. W. Fisher, of the Canada Department of Agriculture, Research Branch, Vineland Station, Ontario, Canada, were of interest for the activity of these polybutenes is based on physical rather than chemical properties.

Briefly, the "Indopol" polybutenes consist of a mixture of butylene polymers - about 85 - 95% being mono-olefines, the rest iso-paraffins. They are non-volatile, strongly viscous even at quite high temperatures, and non-drying: though insoluble in water and oxygenated solvents such as ethanol, they are miscible in common hydrocarbon and related chlorinated hydrocarbon solvents.

Product Grade	L-10	L-100	H-35	H-100	H-300	H-1500
Mean mol. wt.	300	520	730	830	1100	1800
Viscosity at 100° F, SSU	114	1040	7900	44000	140000	-
Viscosity at 210° F, SSU	40.6	93.8	375	1070	3000	15000
Specific Gravity, 60/60° F.	0.831	0.854	0.871	0.881	0.894	0.910
Flash Point, COC, ° F.	270	275	295	360	480	460

Their important physical property lies in their high viscosity at normal temperatures. The raw polybutene is almost impossible to use but effective water miscible emulsions can be made which may be sprayed or painted on to a plant surface to act as a relatively permanent non-drying tacky layer.

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* Manufactured in America by the Amoco Chemicals Corporation, 910 South Michigan Avenue, Chicago 80, Illinois.

Work first published by Fisher (1959a) indicated that certain polybutenes, formulated as emulsions and used at a concentration of 5% active ingredient, effectively suppressed cucumber mildew (Erysiphe cichoracearum) for over three weeks while the untreated control became heavily infected. There was no evidence of phytotoxicity to the crop. Further experiments (Fisher, 1959b) on the control of mites (Tetranychus telarius) showed that the very viscous polybutene H-1500, applied as a 5% emulsion, killed adult mites, larvae and eggs on melons, lima beans, roses, honeysuckel, peach seedlings and other greenhouse crops. Protection from further infestation was obtained for eleven days.

Similarly, the Insecticide Newsletter, January 1960, p.16, summarizing the work at Vineland, claimed effective control of powdery mildew on cucumber, roses and grapes, and control of two-spotted mite, brown mite and European red mite on apples and peaches, and lecanium scales on plums with "Indopol" polybutenes.

At about this time the speaker visited the U.S.A. and observed experiments on powdery mildew of roses (Sphaerotheca pannosa) in progress at Cornell University under the direction of Dr. John Naegele. The results were most impressive and, with the Vineland work, suggested that an evaluation of the polybutenes in Europe might be worthwhile. Accordingly, after discussions with the Amoco Chemicals Corporation and subsequent correspondence with Dr. Fisher, a limited trial programme was begun in 1960 and continued throughout 1961 to test the efficacy of various grades of "Indopol" polybutenes under U.K. conditions.

Extension experiments 1960

By 1960, Fisher's work had indicated that the polybutene grades L-10, H-50 and H-1500 were promising. Later that year further tests showed grades L-100, H-35, H-100 and H-300 to be still more effective - even at concentrations as low as 1%, although strengths of 2 - 3% were necessary where an infection or infestation had become severe.

Since time was short we decided to use grade H-1500, initially formulated as a 50% emulsifiable concentrate but later the concentration was increased to 75%, on apples, hops, potatoes and sugar beet. In all cases applications were made at concentrations equivalent to 5 or 3% active ingredient. During the latter part of the summer L-100 was also tested on hops at a 3% concentration.

Summary of trial results 1960

Apples

H-1500 used at a concentration of 5% gave effective control of Metatetranychus ulmi and several different aphid species on Worcester and Cox.

Sugar beet

At the same concentration an effective control of Myzus persicae was

obtained, thus reducing virus yellows to an extent comparable with demeton-methyl and dimethoate treatments. The control was superior to that obtained with phosphamidon.

Potatoes and Hops

At both 5 and 3% concentration of active ingredient effective control of aphid (Phorodon humuli) on hops was obtained, the foliage comparing favourably with that in the demeton-methyl treatments. The fungicides applied concurrently appeared to stick better on both potatoes and hops in comparison with the control plots to which no polybutene had been applied. As Fisher's latest work also showed that L-10, L-100 and H-50 were useful materials, a 75% emulsifiable concentrate formulation of L-100 was included which, used at a concentration of 3% a.i., showed promise as an aphicide.

Extension experiments 1961

During 1960 work in Canada progressed and more information was obtained about the use of these materials. These results, taken in conjunction with those obtained in this country, led to the initiation of a comprehensive experimental programme for 1961 both in this country and in Europe. Arrangements were also made to obtain toxicological data and to note the effect of these materials on predators.

It was decided to apply for approval through the Agricultural Chemicals Approval Organisation and, as a result independent trials on a wide variety of crops were carried out by the National Agricultural Advisory Service within the terms of the new scheme. In addition, independent trials were arranged at the Glasshouse Crops Research Institute and East Malling, and with collaborators in France and Holland. Most of our own trials in this country were confined to apples. Simultaneously, toxicological evaluation of the four grades under test was initiated at the Nutritional Research Unit at Huntingdon.

Summary of trial results 1961

U.K.

Apples

Berk trials with polybutenes L-100, H-35, H-100 and H-300

Site 1: Essex

Situation

Site aspect: Hilltop farm - gentle slope, frost free.
Soil: Boulder clay predominantly, with some gravel.
Soil cover: Arable.
Drainage: Generally satisfactory but poorer where L-100 and H-35 applied, though not affecting growth.
Variety: Cox/Scarlet Pimpernel; bush trees, age 4-5 years.
Tree health: Excellent - no scab; a little mildew on Cox; no phyto-

toxicity; very good extension growth particularly on Scarlet Pimpernel.

Pest status: Red spider mite - major pest; other pests present at a low level typical for "new" orchards.

Predators: Few present at the time of first spray; increased in numbers later on in the season.

Observations

1. Weather at time of spraying: Fine and clear on each occasion with still conditions so no drifting occurred. Weather remained fine for several days after spraying.

2. Red spider mite: Seasonal development. At the beginning of the season there was quite a high level of infestation. The winter egg hatch was early but comparable with adjacent orchards. First application was thus slightly earlier than usual, but the second one made at the time generally advised (mid-June). There was thus a longer time for build-up to occur. Full results of the spraying programme are given later. However, the effect of the polybutene formulations on the mites was briefly:
Adults - immobilized by the spray deposit and killed.
Eggs (summer) - those hatching within 7 - 14 days of spray deposition were "immobilized" while some inhibition of hatching also seemed to occur.

3. Predators: Polybutenes appeared to exercise no adverse effect on anthocorids and Ladybird beetles seen throughout the trial, while Black-kneed Capsid was observed by June 14th. All became more numerous.

4. Fruit and Foliage: No phytotoxic symptoms occurred on either variety.

5. Mixing in: This was very good for all four grades with H-100 and H-300 being rather less easy than the other two. A 50:50 initial mix for the H-100 and H-300 enables an excellent dispersion to take place.

6. Compatibility: All four grades produced an excellent dispersion when mixed with the standard pesticides and fungicides.

7. Disease incidence: No difference apparent between trees receiving polybutene formulations and those not sprayed with an acaricide or where demeton-methyl was applied.

Results

%		L-100 1	L-100 2	H-35 1	H-35 2	H-100 1	H-100 2	H-300 1	H-300 2	Demeton methyl 12 fl.oz/ac.	Untreated -
May 14th (pre-spray)	M:	607	543	814	666	623	649	702	531	609	668
	E:	52	49	98	83	41	41	104	67	59	44
May 20th	M:	130	66	200	151	52	48	60	35	21	751
	E:	41	20	77	102	62	50	57	45	37	205
May 28th	M:	121	41	72	31	20	11	29	5	32	1004
	E:	32	41	101	27	56	89	30	14	27	196
June 13th (pre-2nd spray)	M:	204	109	121	49	48	31	59	19	41	1302
	E:	63	42	77	33	12	31	11	16	22	205
June 25th	M:	40	31	35	20	16	19	12	12	3	2109*
	E:	45	37	25	21	6	6	3	5	21	285*
July 1st/ 2nd	M:	62	41	71	27	30	21	22	17	10	605
	E:	59	60	30	41	22	15	11	14	16	93
July 8th/ 9th	M:	103	62	114	52	32	19	18	23	25	542
	E:	69	71	39	44	33	27	31	17	21	107
July 15th	M:	171	98	119	79	42	32	34	22	29	311
	E:	83	66	47	36	53	41	27	29	39	88
July 23rd	M:	180	102	63	102	52	44	51	34	35	109
	E:	63	49	37	45	76	55	33	41	37	75
Aug. 9th	M:	253	200	84	98	80	39	69	59	71	291
	E:	71	53	52	74	41	52	45	33	49	112

%		H-100 2	H-300 2	H-300 2	H-100 1	H-100 2	H-300 1	H-300 2	Demeton methyl 12 fl.oz/ac.	Demeton methyl 12 fl.oz/ac
Aug. 20th	M:	41	62	15	22	4	15	9	22	12	42
Sept. 3rd	M:	55	42	12	3	13	7	11	10	6	5

Spray applications: May 17th when winter egg hatch approximately 90%. Second on June 14th: Applied by Drake & Fletcher knapsack.

Mite counts expressed per 50 leaves.

*June 25th: Predators active. Together with mite dispersal results were somewhat masked subsequently. By August 20th impossible to make summer egg counts. Winter egg laying had also begun.

Site 2: Essex

Situation

Site aspect: Valley site - free air movement, with little or no frost risk.
Soil: Brick earth with some gravel.
Soil cover: Arable.
Drainage: Good.
Variety: Cox/Worcester Pearmain; bush trees, 4 - 5 years.
Tree health: Generally excellent; but early in the season most trees were suffering from some growth depression owing to continued wet during the winter of 1960/61. Extension growth excellent for season. Mildew initially widespread on Cox. Scab-risk in area normally low but during 1961 some increase occurred.
Pest status: Red spider mite - major pest; other pests generally present at a low level.
Predators: Few present at time of the first spraying. Increase in numbers later on in the season.

Observations

1. Weather at time of spraying: On both occasions fine and clear with no wind. Weather remained fine for several days after spraying.

2. Red spider mite: Seasonal development: At the beginning of the season there was quite a high level of infestation, with a winter egg hatch of 85 - 90%. The first spray was applied sooner than usual because of an early hatch. The polybutene exerted the same type of control as at Site no. 1.

3. Predators: As at Site no. 1. these appeared to be unaffected by the polybutenes. From mid-June predators became much more active, especially on the untreated control trees.

4. Fruit and Foliage: No phytotoxic symptoms observed on either variety.

5. Disease incidence:

Mildew: It is possible that H-100 and H-300 caused a slight depression in the level of infection but a true assessment will have to wait until "green cluster" stage 1962.

Scab: Trees receiving polybutenes responded normally to the routine scab programme. Phytotoxicity was not observed on either variety.

Results

%		L-100 1	L-100 2	H-35 1	H-35 2	H-100 1	H-100 2	H-300 1	H-300 2	Demeton methyl 12 fl.oz/ac.	Untreated
May 7th (pre-spray)	M:	841	703	887	490	547	907	829	755	1233	809
	E:	15	22	24	11	20	24	27	30	43	14
May 21st (1)	M:	156	83	98	63	31	44	56	29	21	906
	E:	69	104	37	32	49	75	3	5	11	276
June 4th (1)	M:	241	131	133	103	82	70	69	37	39	1096
	E:	77	109	72	46	61	49	22	15	48	342
June 18th (2)	M:	65	49	57	93	25	30	21	13	29	1401*
	E:	40	21	41	5	21	17	41	12	32	141
June 19th (1)	M:	204	102	94	104	59	77	86	63	77	1627*
	E:	56	93	89	21	16	24	42	3	7	256*
June 25th (1)	M:	204	251	150	111	102	61	215	49	114	825**
	E:	78	25	61	49	35	32	41	51	38	103
June 25th (2)	M:	131	98	147	171	47	51	27	21	30	1602
	E:	53	49	104	61	35	13	48	30	30	368
July 9th (2)	M:	198	88	194	132	101	69	68	39	32	1542
	E:	175	92	201	75	25	41	17	20	31	409
July 23rd (1)	M:	190	80	59	35	215	88	163	121	125	633
	E:	82	102	162	98	72	93	62	101	107	33
July 23rd (2)	M:	172	112	182	100	88	75	52	10	26	1011
	E:	211	153	73	88	42	21	41	21	15	207**

M = mites; E = eggs.

Spray applications: May 11th when winter egg hatch 85-90% (1).
7th June (2).
(1) = one spray; (2) = two sprays.
Counts expressed per 50 leaves.
All sprays applied through knapsack - good cover obtained.
* Predators active.
** Thereafter predation and mite dispersal factors operated more noticeably, and masked results.

Site 3: Cambridgeshire

Control of red spider mite on Cox in the Wisbech area was similar to that obtained at Sites 1. and 2.

Other sites

Trials in Kent on three commercial orchards gave rather disappointing results. In part these could be attributed to the grade of polybutene

used and the low rate of application, and also to formulation breakdown and inadequate spray technique. At one site another factor may have been the prolonged egg hatch.

Seasonal development:

As the preliminary results at Sites 1 - 3 seemed encouraging a further series of trials were made to establish whether it was the concentration of polybutene in the spray or a definite quantity of polybutene applied per unit area that mattered. Although more work is needed the results so far indicate that the latter is the more important. However, a good cover must be obtained, and with the minimum of run-off.

Other trials also confirmed that L-100 and H-35 were less effective than H-100 and H-300 in controlling red spider mite, even when used at three times the quantity of the latter materials.

N.A.A.S. trials with H-100, formulated as a 75% emulsifiable concentrate.

East Anglia - winter egg trial:

Spray made on February 20th on twigs heavily infested with winter eggs.

At 1.5% H-100 there was a slight effect on hatching but not really ovicidal.

Kent - on Cox:

Egg and spider counts both higher, especially eggs, than control.

Northern Ireland - 0.75% H-100 on Worcester Pearmain applied in May. By mid-June the control was bronzed but treatments satisfactory - healthy foliage and no evidence of mite attack. By mid-July, re-infestation showing but mites still only about one-seventh of the control population, on a per leaf basis. By late July, a second spray was made. The effect of this on winter egg deposition will be assessed later.

West Midlands - on Lane's Prince Albert:

Red spider mite well controlled by a high volume spray of 0.75% H-100. Control comparable with demeton-methyl.

Cucumber - Red spider (Tetranychus telarius)

Glasshouse Crops Research Institute, Littlehampton.

Polybutenes L-100, H-35, H-100 and H-300 were used at either 0.75 or 1.5% on plants artificially infested with red spider, and compared with two controls - an untreated one and 1% petroleum emulsion.

Three applications were made and, although it was not possible to assess yields owing to a severe attack of mildew on all but the petroleum-treated plants, the trial showed that all four grades of polybutenes at both concentrations were as effective as 1% petroleum emulsion (0.65% petroleum). Slight scorch was caused by 1.5% H-300.

A further trial with L-100 is still in progress. At 0.75% it is giving

a satisfactory control of spider but either alone, or with dinocap for mildew, L-100 is more phytotoxic than certain other compounds except for petroleum. Final results are still to come.

N.A.A.S., Eastern Region - trials with H-100.

Phytotoxicity: Young plants (6-7 leaves) were sprayed to run-off on both surfaces. No damage was observed and the leaves were still tacky two weeks after spraying. At 1.5% and 3.75% the H-100 was also satisfactory.

Mite control: 1.5% H-100 sprayed to run-off. Compared with a control of 1% white oil. The plants were full grown and cropping had begun.

Assessments were made 5 days after spraying using the leaf disc technique when the numbers of eggs, live and dead mites were counted. Egg viability was also recorded.

Although a rather small mite population (350-400 per 20 discs), the results for the white oil spray were similar to those obtained on many other occasions, and in this one small trial there was no evidence that polybutene was in any way better.

In a similar unreplicated trial on another nursery, plants showed severe leaf scorch a few days after spraying; subsequently most of the leaves died and the plants were severely affected. The plants had been sprayed with white oil about 10 days earlier and this also was used on the other plants in the house soon after the trial. Possibly traces of oil on the leaves or drift from the latter spraying proved incompatible with the polybutenes. The plants later recovered and continued to grow normally, but the damage rather took the grower by surprise.

Further observations on leaves and glass plates sprayed with 1.5% polybutene showed that any mites present on leaves at the time of spraying seemed to be killed, but if much webbing is present the mites underneath appeared to be unaffected. Mites moving on to sprayed surfaces do not seem to be affected until they come into direct contact with droplets on the webbing or leaves. On plates, the same thing was noticed, mites being able to move normally on the plates sprayed evenly with 1.5% H-100 but not at 3.75%.

In a third replicated trial, using single plant plots, mite mortality was again about 50% of the total population.

However, these results show only the short term effect of single sprays, and a full programme using these materials might give much better results.

N.A.A.S. Northern Region - trials with H-100

In an observation trial, using H-100 at 0.75 and 1.5%, no phytotoxicity was observed. The 0.75% treatment proved largely ineffective but the higher rate was more successful, comparing favourably with that obtained by azobenzene-parathion smokes in another house of the same nursery.

Sugar beet - Aphis (Myzus persicae and Aphis fabae)

As 2.5% H-1500 had proved comparable with dimethoate and phosphamidon, and only slightly inferior to demeton-methyl, in a 1960 Rothamsted trial, arrangements were made to include H-35 in the 1961 series.

The results were extremely disappointing as, although applied at 3.75%, H-35 proved totally ineffective, which was in marked contrast to demeton-methyl. It would appear that only the more viscous polybutenes might be effective.

Other crops

Blackcurrant - Gall mite (Eriophyes ribis)

Trials were made at several N.A.A.S. centres. Detailed results are not yet available as it is too early in the season for these, but the results so far are fairly encouraging.

N.A.A.S. Eastern Region - trials with H-35.

3.75% H-35 proved non-phytotoxic. Large number of dead mites were present on buds and shoots but final assessments will have to wait until the buds have swollen.

A preliminary bud dissection to assess mite invasion did not give very encouraging results as mites had invaded new buds, although as the level of infestation was extremely high no material could be expected to hold this population during migration.

West Midlands

Preliminary results, where H-35 was used at 0.75% against T. telarius, were less hopeful.

Results from several other centres have not yet been received.

Swede - Swede midge (Contarinia nasturtii)

0.75% H-100 failed to control this pest, the eggs of which are laid in the heart of the plant.

France

Apple

Station de Zoologie Agricole, Antibes.

Preliminary tests show L-100, H-35, H-100 and H-300 to be very satisfactory in controlling red spider. The Braconid, Opius concolor, and several other predators were unaffected. It is not yet possible to distinguish between the four grades.

M. Lambert, Antibes

All four grades were used, and applied three times at 10-day intervals, to the variety Canadian Pippin.

Spider mite was not present but powdery mildew (P. leucotricha) was checked on badly affected shoots. The foliage was otherwise in a very good condition and retained its glossy aspect.

Vine

Station Viticole, Cognac

Against downy mildew (P. viticola) all four grades at 0.75 and 1.5% obtained a control better than 0.5% Bordeaux and comparable with 2% Bordeaux and organo-copper preparations commercially available. Both in efficiency of disease control and persistency the polybutenes were superior to zineb and its analogues, and to maneb. None of the polybutenes proved to be phytotoxic to the foliage or crop. More definite results ought to be available after mid-October.

Other crops

M. Lambert, Antibes.

Carnations/Pinks

Work carried out at Antibes showed that all four polybutenes, used at 0.75 or 1.5%, were phytotoxic; both blooms and foliage were damaged.

Rose

All growth safe when polybutenes applied up to six times normal dosage (0.75%). Excellent control of powdery mildew (Sphaerotheca pannosa) and downy mildew (P. sparsa), and of the mite (T. urticae) and aphid (Macrosiphum rosae) but this incidence was low.

Compatibility

Polybutenes H-100 and H-300 were tested for compatibility with pesticides commonly in use on apples. The tests were made at both high and low volume rates of application with the following results. With high volume applications, both polybutenes were used at 1.5% and the test materials were used at the makers' recommendations.

Miscible liquid/emulsifiable concentrate formulations based on DDT (20, 25%), gamma BHC (20%), malathion (60%), demeton-methyl, and dimethoate were compatible.

Wettable powders containing captan, dinocap, phenyl mercury dimethyl dithiocarbamate (3%), sulphur (75%) or thiram (80%) were compatible. A DDT (50%) formulation was also satisfactory. Dodine acetate (65%) was found to be incompatible. Urea and lead arsenate were found to be compatible.

For low volume applications, H-100 and H-300 were used at 5% concentration in 20 gallons of water.

Miscible liquid/emulsifiable concentrate formulations of DDT 20%, demeton-methyl and malathion (60%) were incompatible. Dimethoate was satisfactory if used immediately, but on standing was incompatible.

Wettable powder formulations of DDT 50%, captan, phenyl mercury dimethyl dithiocarbamate were compatible - indeed, the suspensibility was better where polybutenes were used. Some separation occurred on standing with lime sulphur but the liquids were completely miscible on agitation. A slight build-up of a sticky material occurred on nozzles of the spray equipment, which was presumed to be polybutene.

Mixtures of H-100 and H-300 at both high and low volume concentrations were compatible with colloidal sulphur.

Toxicology

Polybutenes L-100, H-35. H-100 and H-300 both in the raw state and formulated as 75% emulsifiable concentrates were tested for oral and dermal toxicity on rats. These tests showed quite clearly that all were non-toxic even at doses as high as 80 ml. polybutene per kg.

Discussion

The results of our investigations to date enable the following conclusions to be drawn.

1. Toxicity

Polybutenes can be regarded as non-toxic to mammals. With the possible exception of cucumbers and carnations, the polybutenes are unlikely to be phytotoxic. Where damage occurs it seems that the formulation rather than the polybutene is responsible, a point which is being further investigated.

2. Biological activity

In general, grades of higher viscosity are more effective and persistent acaricides than less viscous ones. A better control seems to be obtained when the polybutenes are applied at high volume rather than low, but it is probable that effective coverage can be obtained in volumes around 20 and even as low as 2 or 3 gallons per acre. However, as the mode of action of these materials seems to be purely physical, direct application of the polybutene formulation to eggs, larvae, and adults is necessary.

Where a high population exists more polybutene is probably required initially but a lower rate appears to be effective when the population is smaller. Timing of application is critical and for the greatest effect sprays should be applied at the 80-90% hatch stage.

The question of resistant strains of mites does not arise, whilst there appears to be no adverse effect on desirable insects, enabling a build-up of an effective predator population.

Although a good control of vine downy mildew, powdery mildew of roses and apple mildew was obtained at some sites, in other fungicide trials the polybutenes were ineffective and, so far, results are not comparable with those in North America. These variations seem to indicate that further work is necessary to establish the most effective grades and

to determine suitable application techniques.

Practical applications

Four major disadvantages of acaricides currently in use have been mentioned. Formulations based on polybutene are free of these drawbacks for they are non-toxic to operators, leave no toxic residues and have no adverse effect on predators; nor do they encourage the formation of resistant strains of pests.

When applied thoroughly, the control of fruit tree red spider mite is comparable with that obtained by the use of systemic acaricides based on chemical action. The emphasis must be on effective spray technique if adequate mite control is to be obtained.

Formulated as emulsifiable concentrates, these materials are compatible with a wide range of commonly used pesticides and work is in hand to stabilize mixtures of organo-phosphorus and polybutene formulations. There may well be a case for spray programmes where polybutene and conventional acaricides are used alternately, or where mixtures of polybutenes and established acaricides may be more effective than when either are used separately. However, with regard to the latter, some of the advantages of polybutenes when used alone will be lost.

The problems before the chemical industry and the grower, today, require rapid resolution. The intelligent use of control measures based on physically active compounds can do much to help, as, comparable in cost, they offer all the advantages of a new approach based on an old principle.

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SPECIAL FORMULATIONS FOR LOW VOLUME SPRAYING

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1. PHYSICAL PROBLEMS OF LOW VOLUME SPRAYING

In modern spray operations it is desirable to work at as low volume as possible for economic reasons. When using aircraft this is particularly important since flying time is so costly. High volume was used in the past since spraying to "run off" gave a very even coverage although much of the chemical was wasted. Spraying to run off has the advantage that only the concentration of toxicant need be fixed, but when using more costly chemicals and machines more efficient use of the chemical must be made. However, with the use of low or ultra low (1 to 2 gal/acre) volumes come new problems, some of which are inherent and some of which can be solved by the special design of formulations with which this paper is concerned.

Very low volume spraying cannot leave as even a deposit as spraying to run off. Instead of a general wash the deposit is as discrete droplets and this makes it more vulnerable to displacement by rain or by mechanical means. The use of stickers is therefore more important.

In high volume work a mean droplet size may be of the order of 300-500μ. Assuming a mean droplet size of 300μ, if we spray at 100 gallons per acre, we will achieve about 800 drops per sq. cm. on a plane surface or, since the crop may be 5-15 acres of leaf per field acre, about 50-150 drops per sq. cm. of leaf. However, at 1 gallon per acre we would get only 0.5 - 1.5 drops per sq. cm. and therefore a much greater variation of deposit on individual leaves would result. Thus the droplet size must be reduced. If a mean droplet size of 100μ is used we will get 15-40 drops per sq. cm. of leaf. The deposit, although more uniform, will still be discrete since these small droplets will not coalesce. Droplet size reduction, however, brings with it another problem, namely that of the evaporation of the spray before it reaches the crop. The life of a drop in given air conditions is proportional to the square of its diameter, and it is in the size range of interest that the life becomes critically short.

2. REDUCTION OF EVAPORATION

The obvious method of reducing evaporation is to replace water as the carrier by an involatile oil, but a gallon of oil must always be more expensive than a gallon of water and since the object of this exercise was to cut costs, we may lose more than we gain. The risk of damage is also increased since all the cheaper oils are considerably phytotoxic. If, while using water as our carrier, we may, by the addition of a comparatively cheap ingredient, reduce evaporation, and if this same ingredient can stick our toxicant to the leaf to prevent wash off by rain or accidental brush off, we have solved these two major problems at once.

These problems, particularly that arising from evaporation, are particularly important with solid substances which are presented as wettable

powders. With an involatile active ingredient, if present in a concentration as high as 12% v/v in the spray, even the complete evaporation of the water will result in the diameter of the drop being only halved. If the active ingredient were a solid these residual particles would be dry and therefore will neither adhere to the leaf nor spread on contact. The advantage of reducing evaporation is therefore far more important in the formulation of solids than of liquids.

That the problem of evaporation is a real one even on a cool day in this country, is not always appreciated. It may be shown that the rate of evaporation is proportional to the difference in wet and dry bulb temperatures and radiation has little effect. Even in relatively cool humid conditions with the wet bulb perhaps 1.5° C below the dry bulb a 50μ drop would only fall 2 metres before its radius is reduced to half and the particle becomes dry. In arid conditions, with the wet bulb perhaps 15° C lower than the dry, the distance of fall of a 100μ drop would be little more than 1 metre although under the same conditions a 250μ drop would fall nearly 40 metres.

However, if we interpose at the surface of the drop a monolayer of a fatty substance the droplet life may be increased considerably. The best substances for this use are undoubtedly long chain fatty alcohols (Eisner et al., 1960) but we find that their very low solubility makes them difficult to formulate satisfactorily in the presence of absorbent solids. We have, however, successfully shown that a fatty acid may be used to form the monolayer and that it gives adequate protection.

To illustrate both the importance of evaporation and successful control of it we carried out a field experiment in which a copper oxychloride formulation containing the fatty acid preparation was sprayed along side an orthodox wettable powder by a drift spraying technique.

The spray was produced in an airblast machine directed upwards and allowed to drift with the wind. Samples were collected at 10 yard intervals in a grid pattern on young pea plants and microscope slides inclined at 45° to the horizontal. The results of our experiments in which the air temperature was 78° F and 55% R.H. (Δt 6.5° C) have been shown as contour maps (Figure 1), the results being expressed as γ per sample point (i.e. per 3" x 1" slide or pea plant). Thus it may be seen that a usable plume (greater than 5 γ per sample) of about 100 yards can be expected from the fatty acid formulation and only 35-40 yards in the case of the orthodox wettable powder. That this is mainly due to evaporation was shown by the fact that particles were received on black glass plates held by an operator at up to 150 yards in both cases, but in the case of the orthodox material they rolled off as coarse dust and in the case of the fatty acid preparation no such dust was observed. The average size of the spray droplets was about 60μ for the fatty acid preparation and 80μ for the orthodox material. Over seven times the total amount of copper was recovered compared with the orthodox formulation.

The additive which we worked with was simply a fatty acid, stearic acid, dissolved in an excess of a volatile amine. When dissolved in water

a solution of the amine stearate results which has excellent wetting and suspending properties for our active ingredient. On spraying, the amine stearate migrates to the surface of the drop by normal surface activity effect and the amine, being volatile, evaporates leaving a monolayer of stearic acid which reduces evaporation of the water. In selecting our formulant we have used a tower, twenty feet high and maintained at 10 - 30% R.H at 40° C. These conditions may be considered stringent but we feel that they are not totally unrealistic. The top and bottom of the tower are closed by small air locks to prevent "chimneying" of the air in the tower. In the bottom air lock provision is made to hold glass slides, leaves or small potted plants. At the top is a droplet producing device, either a constant droplet device producing discrete single drops, or a spray nozzle similar to that used in the field. Using the constant droplet device we have shown that no evaporation of spray containing the amine stearate preparation can be detected on droplets down to 80μ although 150μ droplets of a conventional spray are almost dry on reaching the bottom. A more graphic picture results when using the spray jet. A normal wettable powder sprayed at ultra low volume gives a rather broad spectrum with a peak at about 70μ. After passage down the tower at 40° C and 20% R.H. the peak is now at about 30μ. However all material below 120μ is a non-adherent dust which is readily blown off the glass surface. When the same active ingredient is formulated with the amine stearate preparation and sprayed through the same nozzle, the droplet spectrum is narrower, due to the low surface tension, with a peak at about 40μ and after passage down the tower is virtually unaltered although some evaporation below 30μ may have taken place. All droplets received at the bottom of the tower are moist and stick to glass.

This behaviour is illustrated in Figure 2.

3. IMPROVEMENT OF RESISTANCE TO WEATHERING

When the droplet reaches its target, its high surface activity prevents reflection and assists spread on the leaf. The rest of the amine now evaporates and free stearic acid is left. This always crystallises quickly and its crystal structure is similar to that of the natural leaf waxes, so that molecular bridging occurs. The bulk of the stearic acid forms a matrix of interlocked micro crystals which enmesh the active ingredient. A firm, water proof adhesion of the latter to the leaf results. Adhesion is notably better to the leaf surface than to glass.

In the case of insoluble stomach-acting insecticides, used against phytophagous insects, resistance to weathering is unambigously desirable. There is doubt whether it is so desirable in the case of protectant fungicides, where some redistribution in rain and dew may be necessary. It has been shown that additional non-ionic wetters may be used controllably to reduce the resistance to rain although there is then a slight loss of anti-evaporation properties.

We carried out a comprehensive test of resistance to rain, using two active ingredients and the leaves of twelve species of plants. Three leaves of each species were sprayed from a spinning disc with each formulation.

One leaf was kept for reference and the other two were subjected to artificial rain at the rate of $2\frac{3}{4}$" in $2\frac{1}{4}$ minutes, falling from 10 ft. with an average droplet size of 4-5 mm. diameter. The proportions of initial deposit retained after artificial rain 1 hr. after spraying are summarised in the Tables below.

Table 1. Active ingredient - N-methyl ∝ naphthyl carbamate

Leaf surface	% Retained (Orthodox W. P.)	% Retained (Amine stearate prep.)
Banana	0	80
Field bean	2	50
French bean	0	80
Cabbage	0	75
Cocoa	0	95
Cotton	0	25
Maize	2	95
Pea	0	90
Rubber	0	95
Sugarbeet	0	95
Tomato	0	100
Vine	0	90
Mean	0.3	81

Table 2. Active ingredient - Copper oxychloride

Leaf surface	% Retained (Orthodox W. P.)	% Retained (Amine stearate prep.)
Banana	0	98*
Field bean	5	50
French bean	5	80
Cabbage	0	10
Cocoa	0	20
Cotton	0	80
Maize	1	100
Pea	0	100
Rubber	0	50
Sugarbeet	10	50
Tomato	0	75
Vine	1	50
Mean	1.8	64

* Microscopic examination showed some erosion allowed for.

Estimates of the proportion of deposit retained after the application of artificial rain were made visually. We find that the discrete deposits either remain intact or are dislodged as a whole but leaving a visible "ghost" ring where they have been. The proportion of drop residues dislodged can therefore be estimated fairly accurately.

It is of course possible that some invisible erosion occurs, and that chemical estimate of amount retained might give lower values than the visual method. We have therefore made comparisons of estimates of deposit removal in the case of some experimental formulations of N-methyl α naphthyl carbamate, some of which were much less adherent than those finally selected. Good agreement between these methods is shown in Table 3.

Table 3. Estimates of removal by 1" of artificial rain

Visual assessment	Chemical estimation
5%	3%
15%	12%
50%	65%
75%	76%
80%	82%
85%	91%

R. J. Courshee and M. J. Iresan (1961) claimed that 80% of visual assessments are within 15% of chemical estimation in a wider variety of types of deposit.

4. THE FORMULANT IN USE

The amine stearate formulants as supplied are mobile, completely stable liquids. They have low setting points and, if frozen, may be remelted without detriment to their properties. It is necessary to keep containers closed to avoid evaporation of the volatile amine. The formulants are separately supplied, to be mixed in the spray tank with finely ground technical active ingredient, which may contain inert fillers. Any inconvenience in this two-pack supply is compensated by the greater flexibility in ratio of formulant and active ingredient which is permitted to the user.

The use of the formulants with already formulated wettable powders is undesirable. The other wetting agents so introduced may adversely affect reduction of evaporation and always reduce the resistance to rain washing. Also, when the wettable powders contain sodium or calcium salts, the spray liquor may gell or cause nozzle blockage due to the formation of the sparingly soluble stearates. The calcium content of hard waters is not sufficient to cause any trouble in the low volume application for which these formulations are designed.

The formulants are freely soluble in water and promote very easy mixing and good suspension of the solid ingredients. Even at so high a

concentration as 5 lb of copper oxychloride per gallon a very mobile spray liquor is obtained. The spraying properties of the liquors are excellent and the high soap concentration promotes finer division of the spray than is obtained from orthodox spray liquors under the same conditions.

The high concentration of soap does of course facilitate the formation of froth, but the use of excess of the lipophilic amine reduces this tendency and no difficulty need arise if reasonable care is taken to stop air being introduced into the mixing tanks.

The formulants introduce no toxic or dermatological hazards in use.

They are in no way corrosive and indeed have some inhibiting effect on corrosion of metals by other agents.

It must be remembered that they are designed to leave very tenacious residues on evaporation, and therefore unused liquor should never be left for long in spray tanks. In fact the normal hygiene of spray machinery maintenance should be enforced.

5. IMPROVEMENT OF RESIDUAL INSECTICIDAL ACTION

Numerous tests have been carried out in the laboratory and one large scale aerial spraying experiment on pine forest infested with pine looper, Bupalus pinearius. These involved the solid insecticides D.D.T., N-methyl ∝ naphthyl carbamate and dried Bacillus thuringiensis culture, comparing product formulated as orthodox wettable powder and in amine stearate formulation. These experiments are described by Q.A. Geering and J.H. Lloyd (in the press) and illustrative Tables are quoted below.

Table 4. Laboratory spraying on Brussels sprouts (Brassica oleracea var. capitata). Observation of mortality of Pieris brassicae larvae 48 hours after infestation on fresh deposits

Treatments (at 1 gal./acre)		Washed (1" rain)	Unwashed
¼ lb/a.	N-methyl ∝ naphthyl carbamate + 4% amine stearate formulant	80	92
½ lb/a.		76	80
1 lb/a.		80	84
¼ lb/a.	N-methyl ∝ naphthyl carbamate in wettable powder	20	68
½ lb/a.		12	56
1 lb/a.		32	56
Control		0	4
L.S.D. (5%)		33%	

Table 5. Laboratory spraying on Brussels sprouts. Observation of mortality of Pieris brassicae larvae 10 days after infestation on fresh deposits

Treatments (at 2 gal./acre)		Washed (1" rain)	Unwashed
½ lb/a.	Bacillus thuringiensis w.p. + 4% v/v amine stearate formulant	95	100
1 lb/a.		95	100
2 lb/a.		85	95
½ lb/a.	Bacillus thuringiensis w.p.	40	65
1 lb/a.		20	95
2 lb/a.		60	80
Control		25	10
L.S.D. (5%)		24.3%	

Table 6. Laboratory spraying on Brussels sprouts. Observation of mortality of Pieris brassicae larvae 2 days after infestation on fresh deposits

Treatments (at 1 gal./acre)		Washed (2" rain)	Unwashed
2 oz/a.	DDT a.i. wettable powder	40	100
16 oz/a.		52	100
2 oz/a.	DDT a.i. technical + 12% v/v amine stearate formulant	80	72
16 oz/a.		100	96
Control		8	8
L.S.D. (5%)		27%	

Table 7. Field data for aerial spraying of forest of 40 year old mixed stand of Pinus silvestris and P.nigra. Population reduction of Bupalus pinearius larvae corrected for natural population reduction

Treatments (acre-dosage at 1 gallon/acre)		Weeks after spraying						
		1	2	3	4	5	6	7
¼ lb	N-methyl ∝ naphthyl carbamate wettable powder	0	6.4	3.4	0	0	0	11.0
½ lb		23.4	41.3	33.7	13.9	0	0	17.9
1 lb		20.7	45.4	56.2	39.1	21.5	54.2	75.2
¼ lb	N-methyl ∝ naphthyl carbamate + 12% v/v amine stearate formulant	21.5	56.0	57.8	26.0	20.6	55.9	59.4
½ lb		22.3	39.7	41.4	55.9	68.2	63.0	63.0
1 lb		25.2	40.0	51.6	60.1	56.4	49.3	65.1

Notes to Tables

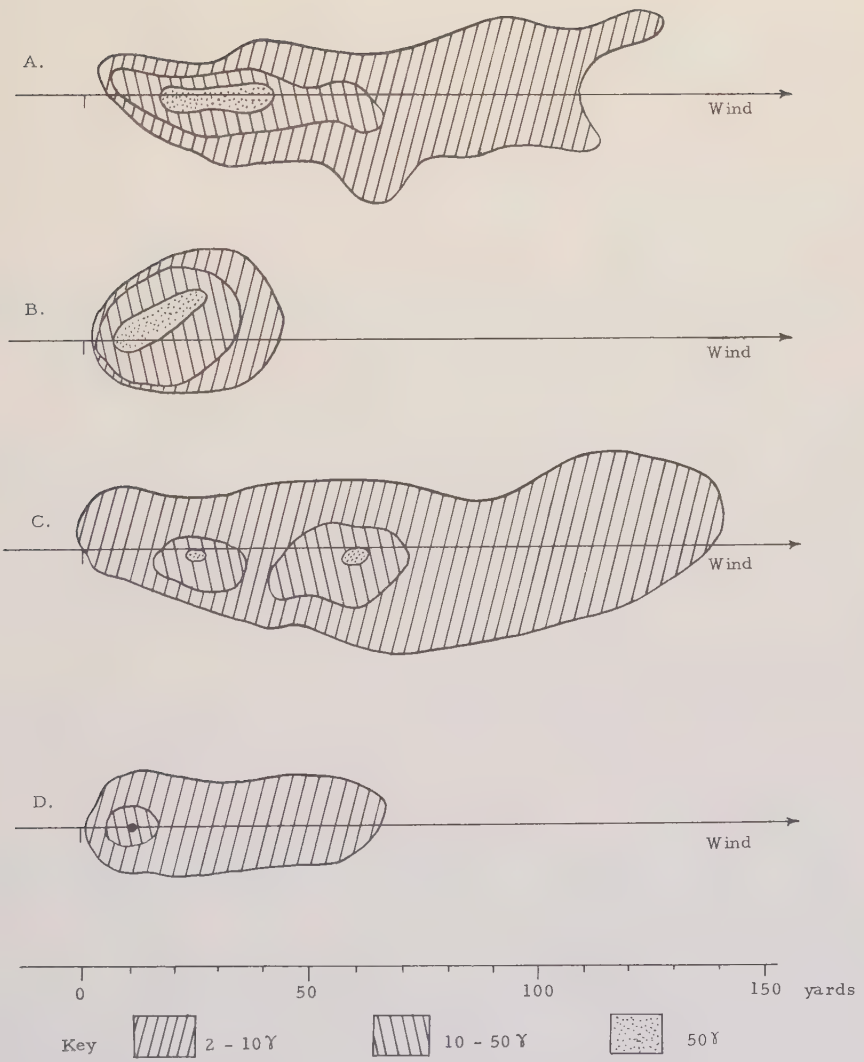
(1) The amine stearate formulation recorded in Tables 4 and 5 was a solution of 61% stearic acid in 39% methyl dibutylamine. That in the other experiments was 22% stearic acid, 14.5% methyldibutylamine and 63.5% inactive solvent to lower setting point.

(2) In some cases it will be noticed that the special formulation is more effective even before washing. This is due to improved initial retention (i.e. reduction of droplet reflection from the leaf.)

References

COURSHEE, R.J. and IRESON, M.J. (1961). J. Ag. Eng. Res. 6(3), 175.
EISNER, H.S., QUINCE, B.W. and SLACK, C. (1960). Disc. Faraday Soc. 30, 86.
GEERING, Q.A. and LLOYD, J.H. (in the press). XIIIth International Symp. on Crop Protection, Ghent, 1961, in press.

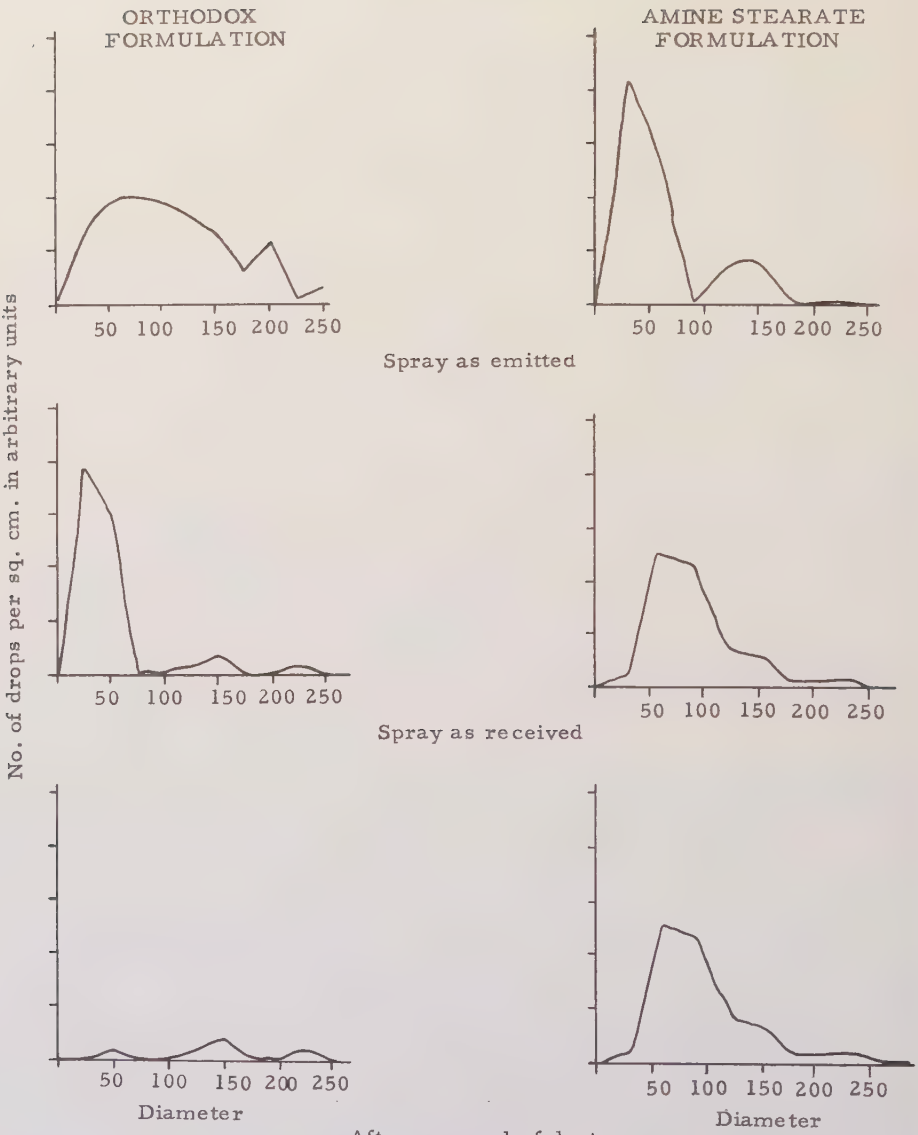
FIGURE 1



Contours of copper deposits in γ per sampling point.

A. Fatty acid preparation on 3" x 1" microscope slides.
B. Orthodox formulation on 3" x 1" microscope slides.
C. Fatty acid preparation on young pea plants.
D. Orthodox formulation on young pea plants.

FIGURE 2


ORTHODOX FORMULATION
AMINE STEARATE FORMULATION
No. of drops per sq. cm. in arbitrary units
50 100 150 200 250
Spray as emitted
Spray as received
After removal of dust by blowing
Diameter

Discussion

Q. Mr. P.G. Clinch

Has Dr. Linke any evidence of the effectiveness of thioquinox against Fruit Tree Red Spider mites resistant to any of the groups of chlorinated hydrocarbon acaracides?

A. Dr. Linke

No. We have no own evidence from trials in this country. However, experiments carried out by colleagues in Italy have shown thioquinox to be effective against Fruit Tree Red Spider mites resistant to "Kelthane".

Q. Dr. H. Martin

Is there any evidence of the spontaneous oxidation, after application, of the sulphide to the sulphoxide and ultimately the sulphone?

A. Dr. Meltzer

We have no definite results of the investigation of the metabolism of the sulphide within the plants, but there is some evidence that after its application there appears some sulphoxide and sulphone in the leaves.

Q. Dr. W.E. Ripper

When referring to the selective action, did Dr. Meltzer mean by insect predators to include predacious mites?

A. Dr. Meltzer

When I am speaking of insects, I really mean insects, and not - like the Americans - all small animals, which are not mammals. But as to the sulphide we have no evidence so far that it is affecting predacious mites. We know that the sulphone tetradifon is completely safe for predacious mites, and therefore we may assume that the sulphide behaves similarly.

Q. Mr. D. Tyson

Do the butylene polymers offer advantages, when used high volume under glass on cucumbers, over the accepted use of white oils for red spider control?

A. Mr. Greenfield

Further work is in progress on cucumbers. Phytotoxicity we have experienced to date has been very slight and the materials are certainly effective against red spider mite. We shall be in a better position to answer your question more fully when we have the results of next year's trials.

Q. Mr. N.B. Van Buren

Could you give us the advantages, if any, of polybutenes over the classic oil sprays?

A. Mr. Greenfield

I think the main point is that there is less likelihood of phytotoxicity using properly formulated polybutenes. However further evidence is needed to enable a complete answer at this stage.

Q. Dr. G.S. Hartley

In the film were the aphids shown in an active state not contaminated with polyisobutene? Was their abnormal activity due to the bright photographic light?

A. Mr. Greenfield

Aphids are normally stimulated by light. The source of activation here was sunlight. The object of the film was to demonstrate the immediate cessation of activity as a result of a high volume polybutene spray, in other words the reverse of stimulation.

Q. Mr. D. Tyson

How residual is the action of Butylene Polymers on crops under glass against spider mite and aphids, i.e. the length of time between sprays?

A. Mr. W. H. Read

The persistence of adhesive action of polybutenes at 5% is up to ten days but these concentrations are highly phytotoxic. At 0.75% the adhesive action does not persist more than 24 hours except on portions of the leaves, e.g. positions adjacent to the veins.

Mr. Greenfield then commented:

We have got Dr. Fisher, who has carried out all the original work, here from Canada and I think it would be useful to have his observations. In our own U.K. trials on apples, cucumbers, sugar beet, blackcurrants, potatoes and hops, we noticed the adhesive effect persisting from 7 - 14 days. I agree that it is only acaricidal for possibly something up to 5 days but we have also noticed that the mites dislike walking over tacky surfaces and, in addition, it tends to dissuade them from laying eggs. I think Dr. Fisher would like to add a comment.

Dr. Fisher then spoke:

Polybutenes used at 0.75% do not last for many days, however, they do control all active stages and if applied at weekly intervals for 2 or 3 weeks, will provide quite good control. In North America polybutenes of lower viscosity have been used because they had to compete with spray oils in cost to the grower. Two sprays of L-100 or H-35 at 2% give good control of European red mite on peach. Bi-weekly application of L-100 at 1% gave me good control of powdery mildew of cucumbers. At Geneva, N.Y., it was found that a residue of polybutene reduced the egg laying of pear psylla to about one tenth, but had no effect whatever on the adults.

Q. Dr. H. Martin

Has Mr. Howes any evidence that the fungicidal efficiency of a particle of copper oxychloride is impaired if it is treated with a film of stearic acid?

A. Mr. Howes

No, we have no evidence to this effect, rather to the reverse. Certainly not that it is impaired in any way but we have in certain trials got better effect - about 30% better - by using the amine stearate formulation rather than a straightforward formulation at high volume.

Dr. Hartley added:

Aqueous hydrochloric acid rapidly extracts copper oxychloride from the deposits, leaving a porous matrix of stearic acid crystals.

Q. Mr. M.J. Way

In view of the fact that the action of systemics like Menazon depends on persistence on the leaf surface, do the Lovo formulations benefit the persistence of systemic residues?

A. Mr. Howes

We really have no information on how it affects systemic insecticides. It may well have an effect on it but in the first case the formulation was designed specifically for active ingredients which are insoluble solids and therefore more difficult to formulate for low volume spraying. We are, of course, open to suggestions and will try this. We have thought that it will help systemic insecticides to get in but we have no evidence to this effect as yet.

Q. Dr. W.E. Ripper

The findings of Dr. Hartley and Mr. Howes seem to conflict with the generally accepted hypothesis that resuspension of copper fungicides in dew was essential for good results of low volume spraying. The work of Kerssen and Courshee showed that rain fastness and fungicidal action were inversely correlated. Are we to deduce that the Lovo compounds lead to a greater fungicidal activity because of larger deposition of fine droplets or is the above cited hypothesis untenable in the face of the new findings reported here today?

A. Mr. Howes

Actually, we have not found this effect which you reported. We find that provided the copper reaches the leaf and actually does stick to the leaf we do get fungicidal activity. We have, however, bowed to this belief; as I said at the end of my paper, we have incorporated a permanent non-ionic wetter in our formulation for fungicides, to promote redistribution. Indeed, we can get quite considerable redistribution and yet still approach the results achieved at 20 - 30 gallons per acre. You get much more shift of the copper, but even when sticking it very firmly as in our initial experiments, using an extremely strong sticker, we have a very

good result. All I can suggest is that the copper is capable of being leached out by water and small quantities which we may not be able to detect are redeposited over the leaf. Indeed, that copper may be leached out with water can be shown by the fact that dilute hydrochloric acid will remove all the copper from the deposit.

Dr. Hartley added:

It is possible that the low volume spray protected from evaporation may convey more very small (satellite) drops to the leaf, which drops would be lost from a normal spray.

TRANSLATION OF RESEARCH INTO PRACTICE - THE INTRODUCTION OF INSECTICIDES AND FUNGICIDES INTO AGRICULTURAL PRACTICE AT HOME AND OVERSEAS

Chairman: E. E. Cheeseman
Agricultural Research Council

THE TRANSLATION OF RESEARCH INTO PRACTICE - AT HOME

by M. Cohen
National Agricultural Advisory Service

The foregoing Proceedings have ranged over a wide field of activities covering both research and practice in the fields of development and usage of insecticides and fungicides.

The present paper is an attempt to look at the process of translation of research into practice and narrowly at that, since the terms of reference of the writer were defined in that the area to be covered was to be restricted to the U. K. Even so the covering of the subject in an area where science and practice have worked together, more or less for a long time, is made no less difficult by this very fact. Generally speaking the speed and thoroughness of the adoption of new ideas and advances is very dependent on the enthusiasm of those who make the advances and the receptiveness of those who are supposed to benefit by them. Those who make the advances may be divided very broadly into two groups. First there are those whose sole interest is in the solution of a problem so as to enable the user of the information to obtain better results in terms of quantity and/or quality of yield at an economic cost. The other group has the same object in view but additionally there is the added aspect of financial gain through the sale of products developed to bring about these results.

So far as those who are to benefit are concerned the speed of adoption of the results of research is dependent on a number of factors of which a few will be briefly considered. Thus the size and effectiveness of advisory services, whether they be independent or ancillary to the commercial interests involved, must be of great importance. Again the level of education of the users must influence the rate of acceptance and the thoroughness of practice of new ideas and developments. The agricultural and horticultural communities cover a very wide spectrum of levels of education. They are no different from those engaged in industry in this respect and it is not suggested that they are in any way exceptional. This factor of variability in ability to receive and absorb new ideas is inherent in mankind and in the less well developed countries of the world the task of the adviser is made more difficult by the need to pitch the advice at a level suitable perhaps to peasant agriculture and horticulture where both the development of husbandry practices and education are at a relatively early stage.

The practicability of integrating the results of research into commercial agriculture and horticulture, the activity of governments and agricultural organisations in supporting the adoption of new developments in the interests of nations and individuals, the economic climate of the industry are other factors influencing the process of translating research into practice that will not be considered further in the present context although they are clearly of varying degrees of importance.

Having considered some of the generalities affecting the topic it is necessary to examine it in more detail in respect of insecticides and fungicides. One way of attempting this would be to recount the progress of development of new materials. Briefly the story would proceed as follows. Sometimes a compound of promise is found by accident or is revealed as a result of screening tests in the laboratory. Following on laboratory trials, toxicological work, small plot work and more or less widely distributed field trials together with notification of intention to introduce and possibly approval, it is launched on to the market, sometimes at very great cost and often with considerable effort to impress potential users and advisers with its superior attributes. Consideration could then be given to a number of compounds to illustrate this process which would cover the topic so far as research by commerce is concerned. Reference could be made to examples of developments arising from research in institutes, universities and by independent advisory services and this would cover the remit to deal with the translation of research into practice.

Alternatively the work of the official advisory service - the National Agricultural Advisory Service, the Experimental Farms and Horticultural Stations in taking the results of research to the agricultural and horticultural communities could be described and this too would satisfy the terms of reference of the writer. But both these approaches are well known at least in outline to those who work in commerce, in institutes and in advisory services and there would be little advantage in enlarging on them. It is therefore considered that there is greater merit in examining some of the problems involved in the daily work of those who advise. A further limitation is proposed, namely that the examination be restricted to some of the problems of those whose sole purpose is to advise to the best possible advantage of the user of the advice without any other factors influencing the decisions that are made. In short the remarks that follow are based on some of the experiences of the National Agricultural Advisory Service.

Problems arising from the giving of advice affect both the adviser and the grower who puts the advice into practice. The latter will tend to recognise the extremes of the effectiveness of the advice namely either that it has done all that was claimed for it or it has failed completely to effect the desired results. In general he will tend to be less certain in his opinion about intermediate degrees of success. Therefore to make the discussion of problems cover more than the extremes of performance of materials the following assumptions are proposed. If an adviser recommends a treatment and the grower carries out the treatment adhering as closely as possible to the details of usage, (this is one of the assumptions that the introducer of the material generally has to accept) and if the ad-

viser follows up the effect of his advice, as he often does, then the adviser is in a sense the user of the material involved in the treatment. There are limitations to this thesis but irrespective of these it affords a convenient method of presenting the 'practice' side of this account.

Consider first of all what is available to the adviser as the result of research in commerce and institutes. Martin (1961) in his compilation of the chemicals used in crop protection, lists over one hundred active principles in the field of insecticides and fungicides, principles whose names are more or less well known to those working in these fields. Of these compounds many have been introduced on an experimental or commercial basis since 1941 and some 61 within the past 15 years. This reflects the pace at which research has been prosecuted and the hectic rate at which new compounds have been introduced in the post-war period. Not all of these compounds have come into common use in the U.K. but taking the Approval Scheme list (M.A.F.F., 1961a) as a minimal guide there were available up to April 1961, insecticides based on 28 different active principles, fungicides based on 14 active principles and one nematicide.

Some 19 of the insecticidal principles contained in the list (to the date mentioned) have made their appearance within the past fifteen years and only about 6 of the fungicides were developed in the same period indicating the less successful search for fungicides. The story must be carried a stage further. When the number of active principles is multiplied by individual manufacturers' variations on the theme, the number of proprietary materials available is very large. Using the Approval Scheme list as a minimal measure of what is available, in April 1961 the adviser was faced with some 170 insecticides and over 100 fungicides covering sprays, dusts, smokes and seed dressings. On the basis of averages there were about 6 proprietary makes per insecticidal principle and 7 per fungicidal material and to give examples of the range it extended from one demeton-methyl spray to no less than 17 DDT sprays.

Of course the multiplicity of materials is not restricted to insecticides and fungicides. Since the new Approval List was issued there have been nine supplements and there are now 74 active principles involved in some 500 approved proprietary forms (including the herbicides and rodenticides). This is a minimal measure of what is available. The Report of the Research Study Group on Toxic Chemicals in Agriculture and Food Storage (Appendix B) (M.A.F.F., 1961b) lists 153 pesticides currently commercially available in this country and of these 74 have been approved. Analysis of the figures shows that insecticides and fungicides make up 102 of the total and of these 56 have been approved, so that two thirds of the pesticides available in the country are topical to this discussion and only about half of them have so far been approved.

Undoubtedly the adviser is faced with a plethora of materials and the most he may know about the details of formulation is the content of the active material and the fact that it is for example in miscible or emulsion form. It is considered that knowledge of all the components of a product can be of value to advisers in helping in the quicker elucidation of abnormal performance or explaining varietal susceptibility. A topical ex-

ample of the effect for example of a solvent is that mentioned by Womack (1962) in the field of liquid insecticidal seed dressings. Additionally, complete information on formulation might help to speed up improvements in efficiency leading perhaps to lower concentrations and fewer applications. This is the sort of work being tackled by the Research Stations at Long Ashton and East Malling and by commerce, but over and above this the field experience of advisers could surely help work along. In all this it is not suggested that complete information be available automatically for the farmer and horticulturalist. In the main they are probably not interested and in any case manufacturers would not wish to make complete details available to all and sundry.

Another aspect of the introduction of new materials is the variation in outlook between commerce and advisers during the development stages of new compounds. These differences need not be constant on one side or the other. Commerce may be concerned to find as many possible uses for a compound in one case, and in another effort may be concentrated on its use for the solution of a single major problem. By way of illustration, a multi-purpose insecticide that shows promise will be tried by the adviser to see if it will give better control of one or two pests that are prevalent in his Region e.g. Wheat bulb fly, spider mites, aphids, eelworms. He will perhaps do more work on the compound in the narrower field and over a longer period that those who introduced it. Apart from filling out the details of performance of the compound he may uncover undesirable side effects not shown up in the development work and in this way help manufacturers to amend their views and their labels.

On the other hand compounds may be developed for almost specific use e.g. the control of Apple Scab and Mildew, Potato Blight, Tomato Leaf Mould, the treatment of glasshouse soils for the control of soil fungi and eelworms. Such lines of development may be more frequent where high value crops are involved and often in the case of fungicides. The possibility of control of minor diseases may be less important to the manufacturer and he may not put much effort into research on these aspects. The adviser however is certainly concerned with all diseases that may be affecting the fortunes of his client whether they be widespread or not. He is therefore anxious to examine the performance of purpose-developed materials against a range of diseases on a variety of crops. He is likely to discover new uses for compounds and also uncover undesirable side effects. Examples illustrating contributions made as a result of advisory work are the use of fenson on apples, dinocap on ornamentals and even the long established DDT on Kalanchoe.

At the present time the emphasis on risks, is on safe use as applied to the user and the consumer. Many advisers would also wish that more was known of the performance of materials prior to or at the point of marketing. Clearly if all risks and side effects were to be cleared up before marketing, progress would be a good deal slower than it has been and the costs of introduction would be greater. Perhaps there is no better solution to this problem than to allow practice to fill in the gaps in research at not too great a cost to the user and the seller.

So far the discussion has been limited to what may be termed the ad hoc effects of incomplete knowledge of the performance of materials either as active compounds or in formulation. There is another important effect of the flooding of materials into practice namely that neither producer, adviser or user can keep pace with the output when possible long-term effects are considered. Examples of what is involved here are the problem of resistant races of organisms, the persistence of materials in soils, the effect of residues on beneficial organisms and the effect of materials on wild life. Potter (1961) in his review of the chemical control of insects has covered these points in detail. Jones (1962) has referred to the need to capitalise our knowledge of host/parasite relationships and the writer would extend this to include also the crop protectant. The effects of insecticides and fungicides on ecological complexes is of profound importance and Martin (1962) has reviewed important aspects of the problem with reference to the soil.

Indiscriminate use as a form of insurance against crop loss has recently been much in the public eye and advisers and manufacturers deplore such use. Undoubtedly there is need for more education of those who distribute and those who use crop protectants and above all much more information is required in the field of pest and disease assessment.

What are the real losses caused by pests and diseases and at level of infestation or infection is treatment likely to result in an economic return? For some organisms the risk of crop loss (almost the certainty) and the relative cheapness of avoiding loss coupled with the absence of undesirable side effects made apparent through long usage, are such as to make treatment routine. The organo-mercurial seed dressings on cereals are an outstanding example. But for many pests and diseases this information is not available.

It is not enough to diagnose a condition and advise a programme. What is required is to be able to say in the light of a full knowledge of the biology of the organism and its behaviour under a range of climatic conditions (a) that the level of attack is such as or is likely to develop to such a degree that control measures are necessary to secure an economic return from the crop or (b) conversely to be able to say that control measures are not required. Concisely put this is the acquiring of the ability to relate pest and disease abundance to crop yields.

Is this an idealistic approach? Clearly it could influence the sales and usage of materials if much progress were made. It could contribute much to the expanding fields of farm and horticultural business advice now assuming much greater importance in the present economic climate and likely to be even more important in the light of negotiations to enter the Common Market.

As in many other fields of investigation, reliable information is only obtained at the expense of much time and effort and progress is slow. It involves 'extensive' work (surveys) followed by 'intensive' experimentation under a wide range of conditions. Only a few problems can be investigated at any one time. The advisers in the N.A.A.S. have already made

some useful contributions with the help of their colleagues in Research Institutes and the Plant Pathology Laboratory and Strickland (1957, 1958) has given accounts of some of this work. To show the importance of this line of approach, consider some of the estimates made by Ordish (1952) on information available up to 1947. Thus for all cereal crops grown in the U.K. his figure for the annual loss due to common pests and diseases was something under £8 million. Pest assessment work between 1952 and 1956 has shown that in the oat crop alone the annual loss due to Frit Fly alone amounted to about 250,000 acre - equivalents per year. The immense difference between the two figures is enhanced when it is realised that the U.K. acreage of oats has decreased greatly since 1947. Again Ordish quotes a figures of just over £2 million per annum for losses in the Brussels Sprout crop due to all the major pests and diseases. Pest assessment work between 1946 and 1955 puts the range of annual loss due to Cabbage Aphis alone from £0.5 million to £1.25 million with an average of £870,000. Amongst the diseases the incidence and progress of Potato Blight has been examined by N.A.A.S. field surveys and an analysis has been made of the crop losses incurred. These have been reported by Large (1956, 1958) who showed that in 'Blight' years during the period 1947/56, the potential average yield of 9.6 tons per acre was reduced by the disease to about 8.0 tons per acre.

These examples of real losses due to two common pests and one disease illustrate the importance of this type of work. Obviously there is little economic value in collecting information about pest and disease abundance unless it is related to crop yields and clearly the use of crop protectants ties in closely with these.

One very desirable advance would be the practical development of forecasting. This is available now with some degree of accuracy but only on an area or regional basis for Potato Blight, Apple Scab, Wheat Bulb Fly, Sugar Beet Aphids in relation to Virus Yellows, and possibly Leatherjackets. Even so, general warnings ought not to be interpreted as a signal to commence wholesale control operations since incidence will be influenced by the type and variety of crop grown and local environment and the need for treatment will be determined by experience and local knowledge. An excellent example of the combination of forecasting, timing and the economics of control is that given by Gould and Legowski (1962) for Pea Moth control and it illustrates the approach to the use of crop protectants via pest assessment work. For problems like this and especially for those of wider national importance the adviser is in the best position to make the advances required.

Sufficient has been said to illustrate the problems facing the adviser but there are many others such as the effects of sub-clinical infestation on crop yield, timing and rates of applications, efficiency of cover and of the machines used. The economics of usage are most important because so much of the information available relates to older materials and methods and against a different economic background. The safe use of pesticides containing toxic chemicals is of prime importance and is catered for through the Notification section of the voluntary Agricultural Chemicals Approval Scheme which must be given time to mature before assessing its

success.

The researcher be he in commerce or the Research Institute has contributed and is contributing much to the solution of the problems discussed. Much valuable information is also being accumulated by advisers as a result of the practical use of crop protectants on commercial holdings and over a much wider range of conditions than is generally available to the researcher. Having earlier identified the adviser with the user it is abundantly clear that if progress is to be authentic and speeded up, the traffic in information must be a re-cycling process - from Research to Practice and back again.

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THE TRANSLATION OF RESEARCH TO PRACTICE - OVERSEAS

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You have been exposed for the best part of a week to addresses of a highly specialised nature from this platform and it falls on me to give a much more general paper, on a subject it is difficult to talk on. Much of what I say is self evident, and to many is your daily pre-occupation.

In "translating research to practice abroad" there are four main points I want to make:- 1. The principles are the same whether at home or abroad; 2. Theory and practice should be the same thing; 3. The economics of the subject must be studied and 4. Are we being too complacent? What are we missing?

Taking the first, my colleague Dr. Cohen dealt ably with these principles and mentioned in particular the two-way traffic in ideas, which is particularly applicable to my theme, for as research can learn from practice so can home learn from abroad. We have many distinguished foreign visitors with us who have contributed much to this conference. What is home to us is abroad to them and so I can take the world as my theme. Today we must be internationalists, but agricultural science always has been that; Jethro's Tull's, "Horse hoeing Husbandry" was translated three times into French and pesticide discoveries frequently came into England from abroad. To mention a few:- Tillet's seed dressing, de Bary's work on potato blight, Grison's lime sulphur, Bordeaux mixture, DDT, parathion and so on to the latest compounds mentioned today, to quote but a few.

First of all I want to speculate on the nature of research and I have started by looking up the word in Dr. Johnson's dictionary and find two relevant quotations:- "It is not easy to research with due distinction ..." (Wotton), a remark with which I am sure you will agree and from Glanville "By a skilful application of those notices, may be gained in such researches the accelerating and bettering of fruits, emptying mines and draining fens." All these are sentiments we have much in mind to this day.

When one thought one knew almost everything one also thought it was much easier to translate research into practice, though in reality it was not, and people were surprised when this or that carefully worked out system absolutely cast-iron in the knowledge of the day, did not work.

For instance Tillet in his famous experiment at Troyes in 1750 discovered the cause and cure for bunt in wheat; nitre, salt and lime seed-dressings; but this weak fungicide soon bred out a resistant race of the disease and the attack became bad again. This sort of thing leads to our saying that theory and practice are different things. I do not subscribe to this statement. It's too facile and woolly a remark. If theory and practice do not agree it's because the theory has not been worked out well enough, or in sufficient detail.

Now for my second point. Translating research into practice, particularly abroad, is really reconciling theory and practice and if we are to make theory and practice the same thing then the theory must be as full and complete as possible, and it is when we come to move our home theory overseas that we must look out for and overcome the bigger discrepancies.

We can only peer into the future by our knowledge and analysis of what has happened in the past. Experience has shown me that if I press that button on the wall the light comes on. If I turn the tap marked "C" cold water comes out, theory and practice coinciding. But this does not always happen. If I've not paid the bills it won't happen. Particularly abroad, for in large parts of the world, owing to the strange predeliction the inhabitants have for their own languages, if I turn the tap marked "C" hot water will come out. I have to modify my theory obtained from observation of the facts. This is universal law. This is research. Abroad your home experiences may be reversed.

Now before you translate research to practice you must have some research to start from. Before you expose foreigners to its benefits you must have some idea of whether it is going to be of any use to them or whether they do not know it already. The agricultures you meet overseas vary enormously. In crops, of course, but also in development: they may be very primitive or very advanced.

Research really springs from Baconionism: to quote Sir Francis it is "the uncovering of the secrets of nature".

In translating research to practice we must first of all keep the ends firmly in view. It is very easy not to see the wood on account of the trees. Let us look at these ends, quite frankly; they are:- (1) To reduce losses from pests and diseases, and make agriculture more productive; (2) To increase or maintain the business of the manufacturers of Agricultural chemicals.

These two ends are not always compatible, though they are not necessarily antagonistic: it is, after all, "enlightened self-interest" that has made us what we are today, a densely populated island with a high standard of living compared with most of the rest of the world. This is because we have been able to translate the results of research into practice. The results obtained frequently turned out to be very different from what the discoverer intended, but research has payed dividends.

Let us look at the chain of events leading from two such discoveries. I am taking a very early overseas discovery, and a comparatively recent one. The first one is stepping outside the frame: it is due to Dr. Cohen's referring to me as Atlas. I want to refer to his brother, Prometheus, the inventor of fire. First the legend of fire. Prometheus, wanting to breathe life into man, brought down fire from the mountains. Of course it was most useful, but people were not grateful. The usual inventor's fate befell him, he incurred the anger of the vested interests, and Jupiter had him chained to Mount Caucusus, where a vulture was continuously eating his liver. However in practice this piece of research more than fulfilled

its promise so far, though megaton fire bombs may yet destroy all life.

If Prometheus was disappointed with his results so probably, in the long run, were the French and English discoverers of BHC in the high hopes of their's: it was not the universal insecticide it appeared to be, it smelt, the insects fought back in that wicked way animals have. ("Cet animal est très méchant, quand on l'attaque il se defend"). I have tried to ascertain the fate of the French and English inventors of BHC; they have not been chained to a mountain and I am glad to say they are both reasonably prosperous. As to their health, I believe they tend to suffer from that nervous tension associated with business cares in the chemical industry, with ulcers eating the stomach rather than vultures the liver.

That old cynic, Humboldt remarked that there are three stages in any great discovery, firstly to deny its existence, then to say it was not important, finally to attribute it to somebody else. However apart from this what usually happens today? Some new pesticide is discovered, either through years of patient fundamental research, screening thousands of compounds or because someone has picked up the wrong bottle or used a Fahrenheit thermometer instead of a Centigrade one and done it by this or a similar accident. The Research Department does this. They are supposed to be long-haired boys in back rooms; though they are directed by egg-heads. Why only these two hirsute extremes should concern themselves with research I don't know. I talk now about different departments. The work may really not be as formalised as this and all done on the back of an envelope, but it is done this way even so. The product is then passed to the Development Department, who patent it if possible and look for home and overseas markets. They must find out if it is safe to user and consumer of the produce sprayed. Then the commercial Department must get to work. Not much is heard of this, but it is really the most important of the lot. They shade into the Development Department of course - they must study the product's economics. How much can they get for it? How far can it be tied up in patents, and in how many countries? How profitable will it be? What is it going to be called? Publicity department are also important: they must name the product. One can make big mistakes in naming products for foreign markets; I have known cases where at the best the trade names were unpronounceable in the foreign language and at the worst were downright indecent.

At some point in this chain will be a tiresome and interfering body charged with setting some sort of official approval on the product. It exists to check up on the claims of the manufacturers and it may frequently appear to you to be very irksome as in most cases it is compulsory. In Britain we tend to do without it as a system of voluntary control works very well. You Chairman and Dr. Cohen has spoken of this, though doubts are arising as to whether the position with regard to pesticide residue in food is adequately controlled and some further interference may be needed.

Abroad not the same reliance is placed on the voluntary principle. There is usually either no registration scheme at all, or one in which official approval must be obtained before sales of a new pesticide can be

made. The first situation puts a great responsibility on the manufacturer, if he is a responsible one, as ethically he should see that his product is safe to use under a large number of varying conditions in possibly a distant country. This is expensive work and he must decide whether to take a risk on some new product or indulge in the expensive work of testing.

Consequently the alternative system, that of obtaining official approval of a product may not appear so irksome when the above mentioned alternative is considered: it does take the responsibility away from the manufacturer, though this registration will increase the work he has to do and thus reduce his profits.

Obviously close co-operation between manufacturer and respective government testing station will expedite the work. The larger countries are concerned to know if the material is safe and effective: the smaller also but they usually lack facilities for testing. They will also tend to consider the foreign would-be importer as large and wealthy and may give the impression that all they want is a substantial registration fee. I think this fee is justified even if they do but little testing. Such a country may be comparatively poor and this fee is at least an earnest that the importer has some faith in his product, that he considers it will be of some use and seriously intends to develop it.

Translating research into practice is in effect sharing the benefits of new knowledge. It is an economic activity and this is my third point. It means you must consider it in economic terms and should study your potential market in these terms. Your product will rise or fall, by the economics of use. We in the British Government's new Department of Technical Co-operation are making surveys of this nature; we have recently published one on central Africa and have others in hand. We try and find out the present, potential and future usage of pesticides, the damage pests do and the benefits pesticides, or other methods of control, can give. If surveys of this nature are not made, either by governments or industry, a great deal of time and effort may be wasted. There is a great deal of interest in such methods of approaching pest problems abroad and I urge this method for your consideration.

A problem which is going to face a manufacturer selling abroad sooner or later is that of the market where the farmers could use a pesticide with benefit to themselves but the business would give no, or very little profit to the manufacturer. For instance sorghum seed dressing in a remote area. This might vastly improve a crop, raise productivity in an area, change the social pattern, but not be worth the manufacturer's while for the small turnover involved and the great distribution difficulties they would experience. What is to be done? There are many instances of this sort of thing. It seems unfortunate if a primitive community cannot benefit because the business of supplying them is not profitable. I suggest that the manufacturer who finds such an instance in his surveys should draw the attention of governments and international bodies, such as F.A.O. and United Nations, to the possibilities. This is in essence the problem of all primitive communities, how can they be helped to help themselves?

There is a great need to do this in many overseas communities where the battle against hunger is by no means won and where the pesticide expert can be of great help.

Now I come to my final point. Let us avoid complacency: what are we missing? Let me give an example. I mentioned the discovery of the insecticidal properties of BHC, a great discovery it was. It was made in 1943 in France and England. But Faraday had actually made the product in 1825. What a long time it took us, 118 years for practice to catch up with research. Moreover the French had used the material in the 1914-18 war, burning it to form smoke clouds, and did not find out any insecticidal properties in it. Surely, as it killed lice, that curse of the trenches, the operators and the smoked, must have benefited greatly from it? Somehow the vital observation was missed. Moreover we knew and had used ortho and paradichlor-benzene as insecticides for years and years: why had not someone tried tacking on a few more chlorine atoms to the benzene ring much earlier than they did? Possibly because the research and development workers were too far apart, as were also the different disciplines: chemists and entomologists did not mix. This idea that research and practice were different things prevailed.

With the examples of Prometheus and BHC before us I put to you that there may well be many discoveries in the notes, laboratories and minds of the research workers available at the present moment which we are not using, and we do not want to wait 118 years to see them developed. I do not think our ancestors were less intelligent than we are. Certain things right under their noses they did not see - what can't we see?

Let me give an example of what I mean, Tillets' remarkable experiment on the cause and cure of bunt in wheat was made in the mid 18th century and the results published in 1751. Some elaborate small plot experiments, repeated for two years, showed the cause to be the bunt dust and the cure, seed-dressing: I now show you some slides.

Tillet proved himself on being a practical man, he would not indulge in "philosophical speculations" as to the cause of the disease, although he noted that the causal dust resembled the dust (spores) in an old puffball. Had he done so he might have advanced crop protection by a hundred years. Is there any "philosophical speculation" we are not indulging in that we should be? There is of course a two way traffic in ideas and we could do well to accept them from abroad as well as export them, as Dr. Cohen has pointed out. There is the concept of "diagonal sciences" of Roger Caillois, where phenomena in one discipline can be explained by observations in another and de Chardin's plea that our ideal should be for not only the research worker, but also the man in the street, everyday to wrest a secret from nature. This after all was Francis Bacon's ideal and it still holds good.

Discussion

Q. Dr. P. Becker

Dr. Cohen has spoken of the importance of the adviser as a liaison between research and practice. This task is being made more and more difficult by the type of common names which are now being coined for the newer chemicals. This started with demeton methyl and dimethoate which, compared with names such as aldrin, malathion, phenkapton etc., are much less easily remembered by the adviser and grower who have a very limited knowledge of chemistry. At this Conference we have heard of azinphos-methyl, thioquinox, metham-sodium, phosphamidon and similar unwieldy names. Surely it is possible to devise simpler ones. I should like to take this opportunity to beg those concerned with manufacturing new common names to pay more attention to the interests of the adviser and user and to produce names which are easier to use and remember.

A. Dr. M. Cohen

I am not really able to satisfy the plea made by Dr. Becker. Advisers are not responsible for inventing common names and many would agree that the list of names grows longer and more confusing. On the other hand, when proprietary names become well known long in advance of the general acceptance of a common name, advisers are put in an awkward position. This happened in the case of demeton-methyl mentioned by Dr. Becker.

There is every advantage in settling accepted common names as early as possible in the history of chemicals. As to who should invent such names, it is doubtful whether advisers would be any better at this than the chemists. Dr. Miller may be able to comment further.

Dr. Miller then stated:

The activities of the British Standards committee responsible for common pesticide names has a continuous impact on my work so far as the Notification Scheme is concerned. I agree with Dr. Becker that some of the more recent names are difficult to pronounce but I do not agree that nowadays new names are not coming forward soon enough.

It seems to me that the B.S.I. committee is finding its principle, that whenever possible a common name should reflect the chemical name of the pesticide and that related chemicals should have related common names, is proving more and more an embarrassment to it. It is a pity more consideration has not been given to the "donamen system" whereby names consist of seven letters, alternating four consonants and three vowels. This system, after allowing for words which are unpronounceable or unacceptable for various reasons in the major languages, gives a potential of seven million names and surely, of these, several thousand names with a chemical flavour could be selected and reserved for pesticides. Wittingly or otherwise, the committee has established several donamen names and, so far as I could see, no one had difficulty with one used quite a lot at today's sessions. I refer to menazon.

The difficulties that have to be overcome before a common name can be finally established are truly immense and it would take quite a time to describe them. One of the major problems is to clear a potential name from conflict with registered names in the major countries. The number of proprietary names already registered, and not always used, particularly for pharmaceuticals, is prodigious.

But the B.S.I. committee is overcoming these difficulties and is well in sight of its goal - to establish a common name for a pesticide before it is marketed. With this happy state, one would not have to spend two or three years using a proprietary name only to have to spend the next two years unlearning the name because, belately, a common name has been established. As I have said, the committee is achieving its aim and I can think of several chemicals which have common names and which are not yet commercially available in this country. Menazon, thioquinox and thiodemeton, discussed at this Conference, are examples.

I am a chemist but I confess I do not attempt to remember the chemical names of pesticides. I try to remember the common names and I always have handy my "Grey Reference Book" should I want to look up the chemical name. This reference book is, of course, the recently published British Standard 1831:1961 on Recommended Common Names for Pesticides.

Q. Dr. William Davies

(1) The number of 'trade names' for chemicals should be reduced and kept at a minimum, e.g. 2,4-D has numerous trade names which seems to me unnecessary.

(2) Research and Practice - Research of necessity involves analysis and fragmentation of a problem or problems. It seems to be no one's business to synthesise new information based on research into practical systems of husbandry. The farmer who makes use of "fragmented" results of research has ultimately to synthesise these into his farming techniques. Research workers and farm advisers must help and should conduct experiments whose set purpose and design is to aggregate and synthesise new knowledge into modified farming systems.

A. Dr. M. Cohen

Dr. Davies has raised a different point from that mentioned by Dr. Becker who was concerned about common names rather than trade names.

I think it is important to remember that crop protection materials contain more than the active ingredient. The method formulation and the concentration are important. In the competitive world of commerce Messrs. X consider it essential clearly to distinguish their product based say on DDT from that marketed by Messrs. Y, and for this purpose trade names are inevitable. The crop protection products industry is not unique in this respect!

Q. A.N. Other

I still feel that a point could be made here. Those of us who advise on these chemicals, and those who use the chemicals, are not really interested a bit in what is contained and what is used to make up the chemicals. What we want is a simple name to remember.

A. Dr. M. Cohen

I think the best that can be done is being done. Reference has already been made to the British Standards list. I doubt very much whether the statement that advisers are not interested in the chemicals themselves is really true. It is important at least to know whether the chemical is a chlorinated hydrocarbon or an organo-phosphorus compound on the score of precautions in use.

Q. Dr. E. Holmes

There are two other aspects of Mr. Ordish's economics which are completely different; cases where even if he worked out all the sums he has talked about, the desirable spraying would not be done. One example is in Ghana. My late Company has done a tremendous amount of work on cocoa capsids in Ghana. Although the cocoa grower does not believe he can afford to use the product which is necessary for the job, spraying is very well worthwhile from the national point of view. Another case is in Uganda. The cotton grower has the money to do the job but not at the time spraying is needed. Here it is first necessary to persuade the Governments, in their own interest nationally, to subsidise or make loans. In Ghana subsidies have helped growers to spray cocoa, and, in Uganda, loans have financed cotton spraying until harvest when the grower has the money to pay - in both cases to the great benefit of the countries concerned.

A. Mr. Ordish

Dr. Holmes' remarks are an example of the complexity of the economics of the subject; in fact they are an example of the point I mentioned where local economics create a situation beyond an individual's powers to overcome; the problem then becomes a national or international one. The two cases Dr. Holmes mentions are cases which could well be followed in other parts of the world and these other cases need bringing to light.